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BRUCELLA INFECTION (INFECTIOUS ABORTION) OF SWINE IN THE PHILIPPINES

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FOUR PLATES

Traum was the first to recognize a specific variety of *Brucella* infection of swine in some hog farms in California. The identity of the responsible organism was later thoroughly studied by various investigators in America and other countries, who recognized it as a distinct variety, namely, *Brucella abortus suis* Traum. In an epizoötic in Denmark (1929–1932) Thomsen(12) prepared an excellent and comprehensive monograph on the various forms of the disease as it occurred at that time in Denmark. Judging from the literature, the infection appears to be well distributed in many hog-raising countries of the world today. Recent progress in the modernization of hog-farming everywhere has placed this disease among those of the most vital importance in the swine industry.

In the Philippines its presence was first suspected in a consignment of breeding sows purchased by the erstwhile Bureau of Agriculture from the Davis Farm of the University of California in 1918. The animals were intended to reënforce the breeding herd of the Bureau at the Alabang Stock Farm. Shortly upon their arrival a number of them aborted, and soon the infection spread to other animals. Were it not for the timely isolation, segregation, thorough disinfection, and disposal of the aborting sows, the entire year's crop of sucklings might have been lost. Unfortunately, owing to pressure of work with outbreaks of rinderpest at that time, these cases of abortion

were not studied in the laboratory to determine definitely the responsible causative factor. Strict sanitary and hygienic measures apparently saved the situation for the time being, and nothing alarming occurred until the outbreaks covered in the present paper. Several years ago a culture was isolated in Japan from the aborted fœtus of a sow imported from Great Britain, but only in 1936 did Nohmi, Katow, and Karasawa(11) recognize their culture as *B. abortus suis* Traum.

MATERIALS AND METHODS

Infection at the Correctional Institution for Women.—In the latter part of 1932 the Correctional Institution for Women in Mandaluyong, Rizal, purchased some breeding pigs from Alabang Stock Farm for the purpose of introducing new blood in their herd. March 14, 1933, Mr. Piatos, in charge of the hog project of the institution, brought to our laboratory dead fœtuses from an aborting sow for diagnosis (Plate 1, fig. 1). Saline suspensions of the spleen, stomach, lungs, and livers of the fœtuses were injected into four guinea pigs. One guinea pig showed diarrhœa and weakness, and was killed May 7. The organs were cultured on Huddleson's gentian-violet agar, at 37° C. for two months, but no growth was obtained. The other pigs were killed and no lesions were found. April 2 another batch of dead fœtuses were received for abortion examination. Organ emulsions were injected into two pregnant guinea pigs subcutaneously and two pregnant rabbits received applications of infused organs in the vaginal cavity. One pig aborted, the other died and upon autopsy showed characteristic lesions in the spleen and liver (Plate 1, fig. 2). Cultures obtained from the aborted guinea-pig fœtuses yielded pure growth, presumably of *B. abortus suis* Traum. This strain was designated as C. I. W. No. 1. The serum of the aborting guinea pigs was strongly positive in plate and tube agglutination tests, in dilutions of 1: 500 and above. For some reason the pregnant rabbits did not abort, presumably due to underdosage or to a less effective route of inoculation.

In view of these findings, arrangements were made with the authorities of the Correctional Institution for Women for permission to test the infected herd. Blood samples were obtained from the ears of the suspected sows and boars for agglutination test. Of nine sows tested, five were found highly positive, two highly suspicious, and two suspicious. One boar was negative. The animals being of breeding stock, it was decided to segregate

them, and regular monthly testing was instituted in order to save those that would recover. Sows that showed no improvement in three monthly tests were destroyed.

The second culture, C. I. W. No. 2, was isolated from sow No. 16, which was sent to the laboratory to be destroyed May 3, 1933. Being strongly positive, the serum was collected for stock positive serum. Suspensions of spleen and lymph glands from this animal were injected into six guinea pigs. June 6, one guinea pig died; one was killed for the purpose of isolating the organisms. Both showed typical lesions of abortion in the spleen and liver, consisting of tuberclelike focal necrosis (Plate 1, fig. 2). One moribund guinea pig, which was bled for serum test, proved highly positive in a dilution of 1: 500. One pig died but showed no lesions. Two other guinea pigs aborted. Pure cultures, presumably of *B. abortus suis*, were obtained from the spleen and liver of the first guinea pig, which was killed. This culture was designated as C. I. W. Strain No. 2.

Infection at the Alabang Stock Farm.—In view of the conclusive diagnosis established in the outbreak at Mandaluyong, the Alabang Stock Farm became the subject of the next inquiry.

In the preceding paragraphs it has been stated that some of the breeding sows at the Correctional Institution originated from Alabang Stock Farm. The first blood test, according to the rapid-plate method, of suspected sows in Alabang, was made April 30, 1933, on sixteen animals. Nine reacted strongly, and were immediately disposed of. Among the remaining seven, three were suspicious and four negative. The tube test caught two more positives of the suspicious group which were included in the list to be destroyed. The results obtained were immediately reported to the Director of Animal Industry who ordered immediate disposal of the infected pigs. Eleven animals of sixteen tested were destroyed. At the present writing the disease in the farm appears to be under perfect control, as a result of systematic routine blood tests and the immediate slaughter of positive animals for the past three years. In the last testing of the herd only two cases were found; these also were disposed of at once.

Organs obtained from the aborted fœtuses from an Alabang sow gave pure cultures, presumably of *B. abortus suis* Traum; these were designated as A. S. F. Strain No. 1. The methods of isolating the organisms were in every respect similar to

those employed in the outbreak at the Correctional Institution for Women.

Isolation of organisms.—In all cases the three strains herein reported were isolated from organ infusions of infected fœtuses, such as are shown in Plate 2, figs. 1 and 2, and the spleen of sick sows or guinea pigs planted on Huddleson's gentian violet agar. The emulsions of the organs were sown on the surface of the media and incubated under partial oxygen tension by placing the tubes in a sealed glass chamber with a lighted lamp allowed to expire. The first cultivation usually was slow, requiring about a month at 37° C.; but once adapted to artificial media, the strains under study never failed to grow on ordinary liver-infusion agar.

BACTERIOLOGICAL AND BIOLOGICAL IDENTIFICATION OF THE CULTURES RECOVERED

Morphology and staining characteristics.—In general characteristics the organisms under study in liver-infusion broth correspond to *B. abortus suis* Traum. They are gram-negative, nonmotile, nonsporulating rods of variable size, but generally larger than the organism of Bang.

Cultural characteristics in different media.—The behavior and cultural characteristics of the three strains in the following media were as follows:

Sugars—

On sixteen different sugars (glucose, mannite, maltose, sorbitol, levulose, galactose, xylose, isodulcite, inosite, dextrin, dulcite, arabinose, raffinose, salicin, lactose, and sucrose) no changes were observed.

Milk—

Rendered alkaline but no curdling.

Beef-liver infusion agar—

Slow and sparse colonies.

Potato—

Turning yellowish brown.

Liver-infusion agar—

Growth is abundant with brown tinge when old.

Liver-infusion broth—

After a week at room temperature a thick sediment collects at the bottom of the tube. (Typical porcine type.)

Loeffler's medium—

Slow and scanty growth.

Lead acetate paper—

Blackened after 48 hours. (Typical porcine type.)

Nitrates—

Reduces nitrates readily. (Typical porcine type.)

Agar stab—

Delicate growth along stab line. No growth in deeper part of the stab.

Gelatin shake—

Gray colonies extending to one centimeter below the surface.

Gelatin plate—

Fine, pin-point colonies appear after three days.

Differentiation from B. abortus Bang.—For purposes of differentiation the three strain, C. I. W. Nos. 1 and 2, and A. S. F. No. 1, were each placed on differential media, together with California No. 80 (porcine) and a culture of *B. abortus* Bang from the United States Bureau of Animal Industry (U. S. B. A. I.) as known controls. This method was used by Huddleson(7) as being specific, and confirmed by other workers in this field as such. The following table records the reactions on these media:

TABLE 1.—*Reactions of C. I. W. Nos. 1 and 2, A. S. F. No. 1, California No. 80, and U. S. B. A. I. on differential dye media.*

Culture.	Growth on differential dye media.				Remarks.
	Thionin agar slant 1:50,000.	Basic fuchsin agar slant 1:25,000.	Gentian violet agar slant 1:100,000.	Liver-infusion broth.	
C. I. W.[a] Nos. 1 and 2, and A. S. F.[b] No. 1 (unknown).	Good----	Inhibited--	Inhibited--	Dense, heavy sediment.	Reactions typical of *B. abortus suis* Traum.
			Controls		
California No. 80 (*B. abortus suis*).	Good----	Inhibited--	Inhibited--	----do----------	Do.
U. S. B. A. I. (*B. abortus* Bang).	None----	Good-----	Good-----	Thin sediment--	Reactions typical of *B. abortus* Bang.

[a] Correctional Institution for Women [b] Alabang Stock Farm.

In Table 1 the reactions of the three swine strains under study in the dye media compared with a known porcine strain, California No. 80, and a bovine strain, U. S. B. A. I., were

recorded. As a result of the tests in the differential media, and the cultural and morphological characteristics in all the other media employed, there is no doubt that the three strains herein reported are all of the porcine type, *B. abortus suis* Traum. Repetitions of the foregoing differential tests have yielded uniformly similar results.

Serum reactions.—It is generally admitted that type differentiation by serological methods is not conclusive, since cross reactions exist between types. Such reactions merely indicate group relationship. The sera of infected pigs agglutinated the antigens prepared from stock cultures of *B. abortus* Bang, and California strain No. 80, of porcine origin. An imported bovine serum from aborting cows likewise agglutinated the antigens from the three field cultures under study. It is quite evident, then, that the bovine and porcine types of abortion can not be differentiated by agglutination.

OBSERVATIONS DURING THE OUTBREAKS

Symptoms and lesions.—In the aborting sows a characteristic white purulent discharge from the genitalia was regularly noted. In one instance a boar showed a pronounced orchitis which became chronic, and upon castration the testes revealed varying degrees of abscessation; another boar was highly positive in the agglutination test, although it appeared normal, and upon palpation the testes were rather indurated. Although the abscesses in the testicles may heal up, other foci may persist, as shown in Plate 4, figs. 1 and 2. It is stated that pure cultures of the organism may be isolated from such abscesses. The infected uteri of sows with the chronic white discharge, upon incision, showed a metritis of long standing. However, in many positive sows and gilts no untoward manifestation of disease was discernible beyond a record of past abortions and poor breeding. A number of sows were known to farrow normal litters after a previous abortion. These observations tally very closely with those of Graham et al.(2) in Illinois.

Organs of predilection.—In pigs actively infected with *Brucella suis* the spleen and liver are favorite points of attack, from which organisms may be isolated. Guinea pigs injected with infected tissue or culture invariably show lesions in this organ, especially in the liver. Here typical tuberclelike nodules appear one to two months after inoculation (Plate 1, fig. 2). The nodules are white, subcapsular, and are scattered in the organ. On section of the individual nodules, a true picture of focal

necrosis may be observed without giant cells. Frequently organisms have been isolated from the internal lymph glands, the uterus, the submaxillary gland, the kidneys, and the afterbirth of the infected sow. The aborted fœtuses have been found in the present study to be a very reliable source for obtaining pure cultures of *B. suis,* because all the organs are saturated with the organisms. Direct cultures from the spleen of freshly aborted fœtuses are not difficult to obtain, as already mentioned elsewhere.

Condition of aborted fœtuses.—In Plate 1, fig. 1, the fœtuses appear normally developed in certain cases, while in others, (Plate 3) there is advanced mummification at the time of abortion. Likewise, some sows abort before the fœtuses are fully developed (Plate 2). Without serum diagnosis it is evident that such abortions may be mistaken to be due to other causes. With the present knowledge of the disease in swine it is obvious that all cases of abortion should be given the necessary serum test to rule out *Brucella* infection. Since there is no way of distinguishing forms of abortion by other means than the serum test, the latter should be adopted as a diagnostic method, especially in localities where infectious abortion in swine is known to exist.

INCIDENCE OF INFECTION IN HOGS KILLED AT THE MANILA ABATTOIR

For purposes of this study, a preliminary survey of the blood of local pigs slaughtered at the city abattoir was undertaken. Of 1,975 blood specimens collected on the killing floor and subjected to the rapid agglutination test, 2.2 per cent turned out positive. For lack of available funds we were unable to secure the carcasses of serum-positive hogs for the isolation of the organism from this source. Furthermore, this work had to be suspended due to the appearance of foot-and-mouth disease in the abattoir. At this writing pressure of other work prevents the continuation of this survey. However, it will be resumed in the near future.

TRANSMISSION OF BRUCELLA ABORTUS SUIS INFECTION AND ITS TRANSMISSIBILITY TO MAN

Transmission in swine.—For the present report transmission experiments were not conducted, but the work of Howarth and Hayes(5), and of Johnson and Huddleson(9) in the United States seems to indicate that the boar plays an important rôle. Milk

of infected mothers may transmit the infection to the young by ingestion, but it is the consensus that the most prolific sources of infection are contaminated feed and water in infected premises, and the infected boar.

The observations of Hayes(4), regarding certain inconsistencies between natural infection and outcome of pregnancy, are rather interesting. He noted that positive sows from the uterus of which *B. abortus* has been isolated may farrow normally. Likewise, sows having positive sera may farrow normal pigs. On the other hand, serum-negative sows may abort or farrow dead pigs. He concludes that a positive diagnosis of abortion infection in a sow does not indicate the outcome of pregnancy. However, the presence of the infection in any herd in whatever form must be considered a potential source of economic loss that is bound to manifest itself sooner or later in the form of failure to conceive, as observed by Johnson and Huddleson, or the more common occurrence of intermittent abortion among the infected sows. The infected boar which develops a chronic orchitis not only may be incapacitated for breeding but also becomes a spreader of the disease to other sows through coition. It is at once obvious that such breeding conditions run counter to profitable hog raising.

Transmissibility to man.—This phase of the swine abortion in the Philippines has not been investigated, and the reports mentioned in the following paragraphs are merely informative in nature.

In the study of Huddleson, Johnson, and Hamann(6) in the Michigan packing houses, some illuminating information may be gained in advance. Of 3,975 hogs examined serologically and bacteriologically, 7.74 per cent were positive serum reactors, and the infective organisms were isolated from 13.31 per cent of the reactor hogs. Simultaneously a study of the blood of 167 employees in four packing establishments and stock yards revealed agglutination reactions in 10.7 per cent of the individuals. The development of a clinical case of undulant fever in one establishment eight months after the first test necessitated a retest of 18 employees who showed agglutination reaction to dilutions of 1:25, 1:100, 1:200, and 1:500. In 100 cases of undulant fever treated with "brucellin," Huddleson, Johnson, and Beattie(8) isolated 16 cultures of *Brucella* organisms from the blood of 85 cases. Of these, 7 were *B. abortus* Bang, 5 *B. abortus suis*, and 4 *B. melitensis*. In a personal communication

to the writer Dr. W. de Leon(1), chief of the laboratories in the Philippine General Hospital, Manila, in his preliminary work on the sera of suspected undulant fever patients in the hospital, reports 12 cases that reacted positively to *B. abortus* Bang antigen in a dilution of 1:100 to even 1:500; 3 of these were aborting women. Isolation of the organisms from the blood of positive patients is now being conducted by Doctor de Leon and his assistants to determine what type of *B. abortus* organism is responsible. I venture to suspect that *B. abortus suis* might be the causative agent in these cases, since pork is preëminently the meat eaten in the Philippines.

McAlpine and Slanetz(10) in Connecticut in studying the glucose utilization of the bovine and porcine *B. abortus* group, including *B. melitensis,* found that all 10 strains of *B. abortus* of human origin, which he tested, were closer to the porcine group than others. Considering the greater virulence of the porcine type in man than that of other types, this finding can readily be accepted without discussion, for the *Brucella suis* organism is known to be capable of invading the host even through the unbroken skin. In this respect it is somewhat similar to *Bacillus pestis* of bubonic plague in man.

Graham and Michael(3) state that from the years 1929 to 1933, 6,708 cases of undulant fever were reported to the United States Public Health Service. The states reporting the largest number of cases were New York, 969; Iowa, 595; Missouri, 533; California, 515; Ohio, 480; Illinois, 407; Kansas, 346; and Minnesota, 295. Of these, Iowa, Missouri, Illinois, Kansas, and Minnesota have swine populations of over 3 million. The above authors are quoted thus: "It would seem that there may be a significant relation between the incidence of undulant fever in man and the size of the swine population."

It may be gleaned from the foregoing observations that people handling fresh pork in abattoirs and packing houses, meat markets, and similar places are particularly exposed to infection, principally through skin abrasion or by ingestion of contaminated food and water. While conditions in the Philippines are such that the pork industry is not yet highly developed, the presence of the infection here and its transmissibility to man calls for appropriate preventive and sanitary measures. From this standpoint alone, regardless of its vast economic significance to the hog industry, porcine infectious abortion becomes a disease of importance in relation to public health.

DISCUSSION

The biological and bacteriological characteristics of the three strains presented in this report definitely show that the organisms in question are *B. abortus suis* Traum. Whether or not the disease in hogs was introduced from abroad or native cannot be ascertained with accuracy, since for many years prior to this study imported hogs have been distributed to the different parts of the country. The fact, however, that the isolation of the organisms was made from pigs of imported breed, favors the view that the disease must have been introduced. Moreover, with the discovery by Howarth and Hayes of the outbreak in the Davis Farm, University of California, in 1931, it may be taken that the outbreak of abortion in Alabang among the stock of swine purchased from the Davis farm in 1918 was really infectious abortion introduced from California.

In identifying these three cultures or strains, only standard and accepted methods have been employed, supported by adequate known controls. The results of differential tests, such as animal inoculation and cultural behavior in liquid and dye media, and others, to which these strains have been subjected, point conclusively to the porcine type of organism.

The symptomatology and morbid changes observed in both sexes of infected pigs correspond closely to and were often identical with those observed by other workers in other countries (Hayes and Thomsen). Likewise, the condition of aborted fœtuses in different stages of pregnancy were similar to those noted by others (Graham et al.).

The fact that the cultures obtained from cases in the two outbreaks presented in this report produced abortion in pregnant guinea pigs is further evidence of their identity as true abortion organisms. Moreover, the sera of these aborting guinea pigs when tested against a known *B. abortus* antigen yielded a high positive titre (1:500 and above). Inversely, an antigen prepared from each of the three strains separately likewise agglutinated a known positive serum from abroad. There is, therefore, no room for doubt that the disease exists in this country, and the fact that it is transmissible to man should motivate concerted action towards effective control and eradication, not only from the standpoint of hygiene and sanitation as pertains to man, but also from its more important economic aspect, namely, its destructive effect on the hog industry.

A partial survey by agglutination of the blood of 1,975 pigs slaughtered at the Manila abattoir showed that 2.2 per cent

of the total were positive for abortion. Whether or not this positivity has any direct relation to the incidence of certain indeterminate fevers reported in this country in human beings, among which may have been undulant fever, has not been actually determined. However, the work of Huddleson, Johnson, and Hamann, in connection with an outbreak of undulant fever in a Michigan packing house, definitely established a direct relationship between the outbreak and the finding of *B. abortus suis* in the fresh pork of those establishments. This relationship was supported by the presence of agglutination reactions in the sera of 10.7 per cent of the employees of one packing house in one test and 60 per cent in another. Again, according to the reports of the United States Public Health Service for 1929 to 1933, mentioned by Graham and Michael, the relation between the incidence of undulant fever in man and the size of the swine population is undeniably significant. The fact that of 16 cultures isolated by Huddleson, Johnson, and Beattie from the blood of 85 patients with undulant fever 5 were *B. abortus suis,* proves conclusively that this type was the cause of undulant fever in about 30 per cent of their cases.

In a personal communication Doctor De Leon of the Philippine General Hospital reported twelve serologically positive cases of suspected undulant fever patients, three of whom were aborting women. Although the isolation and identification of the *Brucella* organism from human patients have not been accomplished as yet, it is reasonable to suspect that the porcine variety (*B. abortus suis*) is the responsible agent in the majority of these cases, on account of the intimate association with, and the handling of pigs by, the people, and the fact that pork is the most commonly eaten meat of the country.

SUMMARY AND CONCLUSIONS

1. Two outbreaks of porcine *Brucella* infection have been described and reported, and three strains of *B. abortus* organisms having the characteristics of *B. abortus suis* Traum have been isolated and identified.

2. The findings in this report would indicate that the disease has been introduced in this country from California.

3. The symptoms and lesions observed in both sexes were characteristic of the disease encountered elsewhere.

4. Transmission in swine is known to occur through the ingestion of contaminated material or even through the unbroken skin.

5. The organisms are commonly found in the lymph glands, spleen, uterus or penis and testicles of the infected animal, while the fœtus may be entirely saturated with them, including the allantoic membrane and its fluid.

6. Isolation of the organism from the tissues of the aborted fœtus and the infected sow was accomplished either by direct culture on special Huddleson's medium according to his technic or by culturing the infected organs of guinea pigs showing lesions of the disease from inoculation of infected tissues.

7. Although a positive serum diagnosis in a sow does not indicate the outcome of pregnancy, this uncertainty is highly destructive to swine breeding and the industry in general.

8. Abortion of sows from *Brucella* infection occurs in all stages of pregnancy.

9. Agglutination tests of the sera of 1,975 hogs killed at the city abattoir showed an incidence of 2.2 per cent.

10. Agglutination tests by Doctor de Leon (personal communication) of the sera of suspected undulant fever patients at the Philippine General Hospital revealed twelve positive cases, three of whom were aborting women.

11. In an infected farm routine serum agglutination test and the immediate slaughter of all positive animals along with thorough disinfection were found most effective in placing the disease under control.

12. *B. abortus suis* is virulent in man and was the cause of undulant fever in about 30 per cent of the cases studied by Huddleson, Johnson, and Beattie.

13. Although the responsible agent has not as yet been isolated and identified from human patients reported by Doctor de Leon, *B. abortus suis* is highly suspected in these cases.

14. The fact that this organism is considered the most virulent of the group and has been shown to cause undulant fever in man, calls for special sanitary handling and disposal of fresh pork as a control and as a public-health measure.

ACKNOWLEDGMENT

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ILLUSTRATIONS

PLATE 1

FIG. 1. Aborted fœtuses three and one-half months old from a Black Poland China sow, Correctional Institution for Women, Mandaluyong, Rizal. (The other two fœtuses were badly macerated and could not be photographed.) Photo by Modesto Bantola.

2. *sp*, Spleen, and *l*, liver of a guinea pig showing *fn*, tubercle-like focal necrosis 69 days after subcutaneous injection of spleen suspension from an infected sow.

PLATE 2

FIG. 1. Aborted fœtuses one month old from Red Berkshire sow, 1934.

2. One fœtus with the allantoic membrane removed.

PLATE 3

Aborted mummified fœtuses two months old from Red Berkshire gilt, Alabang Stock Farm, 1934.

PLATE 4

FIG. 1. Apparently healthy Poland China boar with infectious abortion abscess, *ab*. Its serum is strongly positive.

2. A close-up picture of the infected testicle with healed abscesses and scars, *sc*.

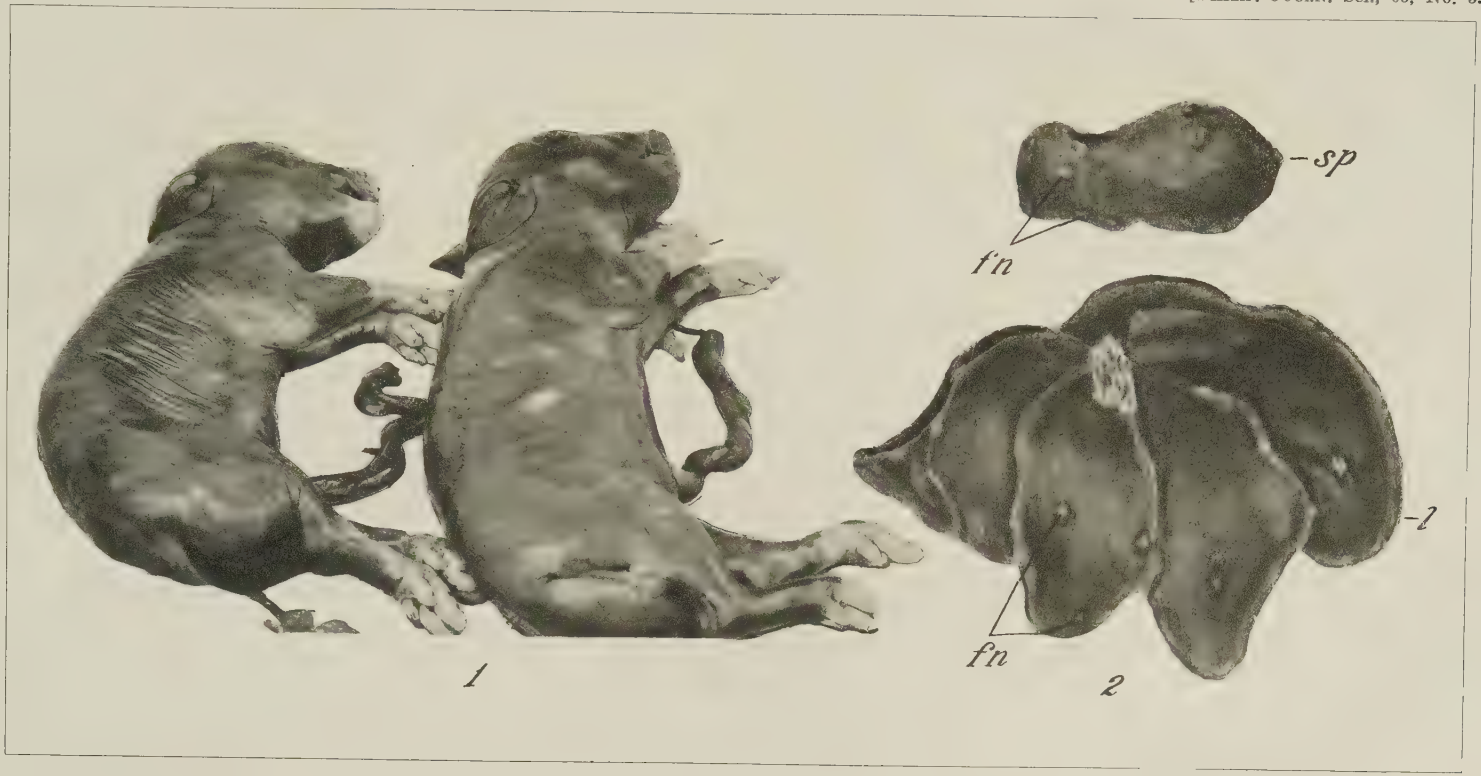

PLATE 1.

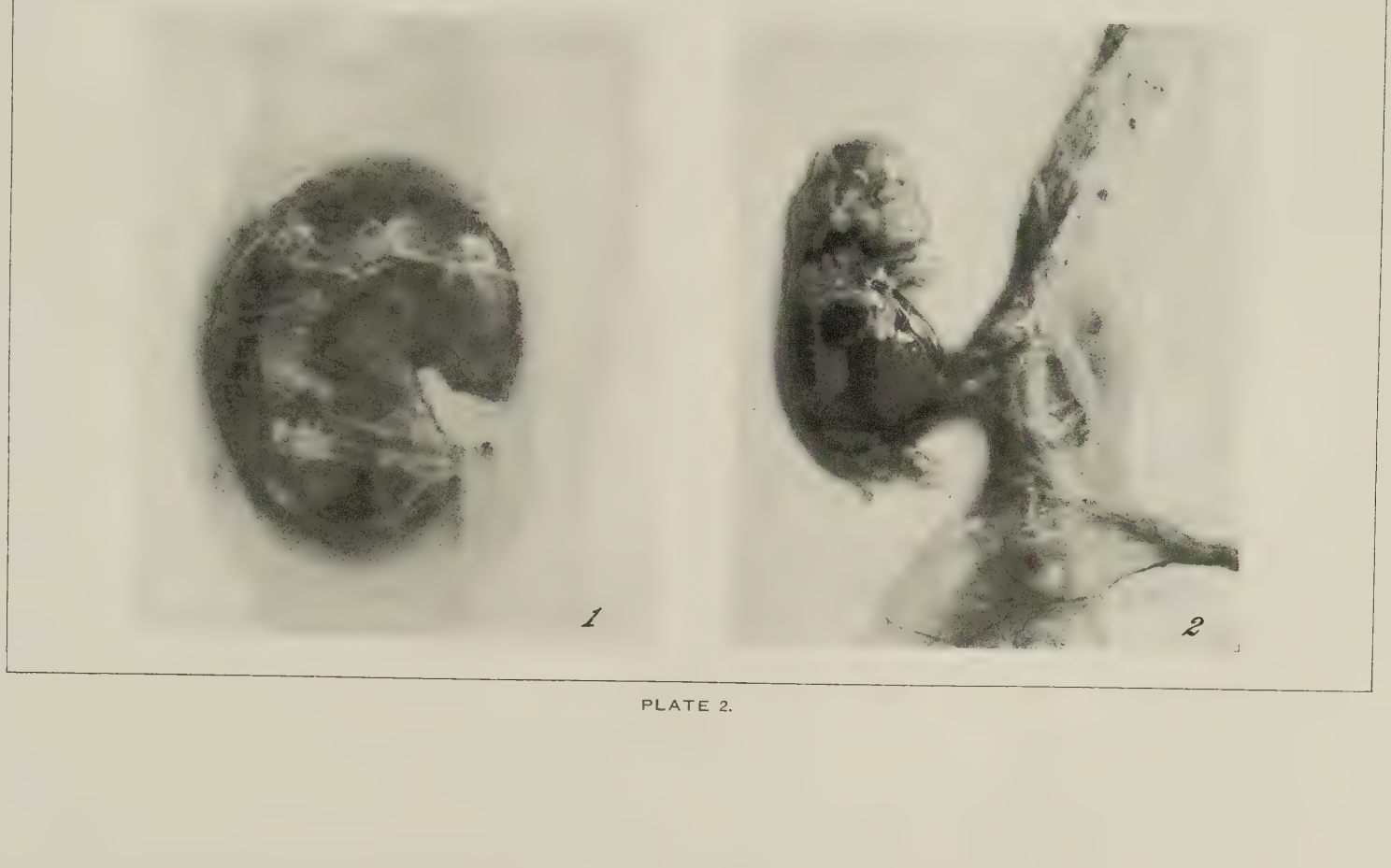

PLATE 2.

PLATE 3.

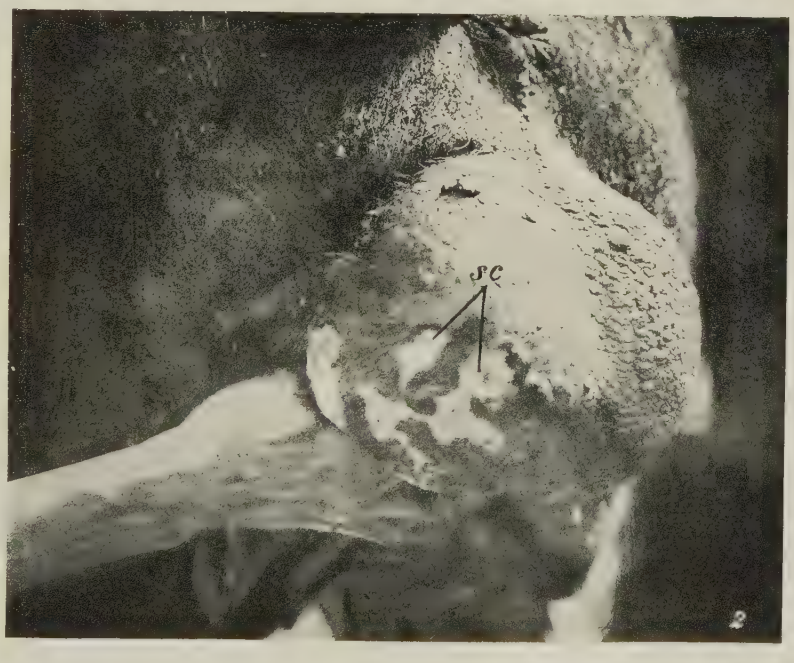

PLATE 4.

A NEW SPECIES OF AMANITA

By José Miguel Mendoza and Simeona Leus-Palo
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ONE PLATE

Formerly it was generally believed that *Amanita* does not exist in the Philippines. Very recently, however, some species of the genus were discovered in high altitudes, as in the vicinity of Baguio, Mountain Province, where a more or less subtropical climate prevails, with high humidity at certain seasons.

Collecting in Manila, January 2, 1936, the senior author and Mr. Buenaventura Reyes found an unrecorded species of *Amanita* in Malate. January is usually a cool month in Manila and at the time of collection of this specimen there were several days of continuous rain. This is the first record of a species of *Amanita* growing in the lowlands in the Philippines.

AMANITA MANILENSIS sp. nov.

Praedita est haec species; pileo succulento et verrucoso qui prima aetate oblongo, serius convexo, aetate vero provecta late convexo, 3.5 cm lato, cano-fuscente ad centrum, subcano vel cano versus marginem; lamellis perpluribus, albis, non affixis; stipite albo, crasso, farto, ad basem bulboso sed gradatim se minuendo ad pileum, 5 mm in diametro ad minimum, 9 mm ad maximum, circa 3.9 cm longo; annulo conspicuo, semper vigente, albi coloris, ad medium stipitis invento; volva tenui, adnata, subfusca, versus marginem inaequali; sporis hyalinis, obovatis, nonnumquam rotundis vel ellipticis, 6 ad 6.60 μ × 5.4 ad 6 μ; basidiis clavatis, granulatis, 21.03 ad 28.5 μ × 6 ad 7.5 μ; cystidiis perpaucis, 30 ad 51 μ × 8.5 ad 10.5 μ.

Pileus [1] oblong convex when young becoming broadly convex when expanded, mouse gray at the center, becoming paler toward the margin, fleshy, thicker at the center; about 3.5 centimeters broad, covered with sharp pointed warts which are denser and larger at the center, becoming sparse and finer toward the margin. Margin at first entire, later cracking with age. Gills free, white, moderately numerous, broad near the margin, grad-

[1] This description is based on fresh full-grown specimens collected January, 1936.

ually narrowing toward the stem, long gills intermixed with short ones. Stem stout, white, stuffed, well formed, tapering gradually toward the pileus from a round somewhat bulbous base, about 5 mm in diameter on the upper end and about 9 mm at the base, 3.9 centimeters long. Annulus very prominent, persistent, white, showing remainder of a veil; located on the midway of the stem. Volva thin, pale brown, adnate, irregular at the margin, covering entirely the base of the stem. Spores white in mass, hyaline under the microscope, obovate, sometimes round, 6 to 6.6 μ long, 5.4 to 6 μ broad; average 6.33 μ long, 5.61 μ broad. Basidia numerous, clavate, granular, 21.03 to 28.5 μ long, 6 to 7.5 μ broad; average 24.7 μ long, 6.72 μ broad. Cystidia very rare, long-cylindric to elliptic, sometimes slightly pointed, 30 to 51 μ long, 8.5 to 10.5 μ broad; average 39.6 μ long, 9.12 μ broad.

LUZON, Manila, *Phil. Nat. Herb. 2582, Jose M. Mendoza and B. Reyes* (type) January 2, 1936, on the ground, under acacia, *Samanea saman* (Jacq.) Merr.

Amanita manilensis resembles very closely *A. aspera* Fr. in appearance. It differs however, in the size of the spores and in the form of the cystidia. In *A. manilensis* the spores are much shorter than those in *A. aspera.* The cystidia in *A. aspera* are subglobose with short constricted base, while in *A. manilensis* they are long-cylindric to elliptic.

Most deadly poisonous mushrooms belong to *Amanita,* such as *A. phalloides, A. muscaria,* and *A. aspera. Amanita manilensis* sp. nov. is suspected of being poisonous.

ACKNOWLEDGMENT

The writers acknowledge their obligation to Dr. A. H. Smith, of the University of Michigan, for the loan of *Amanita* specimens which were used for comparison; to Rev. Francis X. Reardon, S. J., of the Ateneo de Manila, for translating a brief English diagnosis into Latin.

ILLUSTRATION

PLATE 1

FIG. 1. *Amanita manilensis*, a section of the gill, showing the hymenium with cystidia, basidia, and spores; × 667.

2. *Amanita manilensis;* × 1.7.

PLATE 1.

HERPETOMONAS MUSCARUM (LEIDY) IN LUCILIA SERICATA MEIGEN [1]

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FIVE PLATES

Herpetomonas muscarum (Leidy), which is commonly found parasitic in the digestive tracts of nonbiting flies, is one of the most extensively studied flagellates. An important problem is the relationship of the parasite to the larva and pupa of its host. Patton stated in 1910 that he could not find the parasite in larvæ living in horse dung. In 1921, however, he reported larvæ infected with *Herpetomonas muscarum*, in which the infection carried over to the adult. Becker (1923) was unable to find larvæ infected with parasites, both in attempts at experimental infection and in examination of larvæ taken from exposed places in Westport dump, Baltimore. MacKinnon (1910) observed infected larvæ of certain dung flies.

Below are reported studies of the morphology and life history of the flagellates, as well as the results of experiments on infection in adult and larval flies.

MATERIALS AND METHODS

Materials.—The flies were collected by the writer between September and March by means of fly traps. By using as bait under the trap a piece of fish or meat, it was possible to catch in a few hours various kinds of flies, among which were a great number of *Lucilia*. A bottle with a narrow opening was placed inside the trap, and the flies desired were caught by hand and dropped into the bottle. By the use of traps and fly cages much time was saved as compared to that required in using the net.

The larvæ were fed liver, hamburger steak, horse meat, yolk of egg, cooked fish, and molasses, and the flies were given ba-

[1] The observations and experiments reported in this paper were carried out in the Department of Zoölogy of the University of California at Berkeley. The writer wishes to thank Dr. Harold Kirby, Jr., under whose direction the work was undertaken, for his advice and criticism on the manuscript and experiments, and to acknowledge his indebtedness to Dr. Charles A. Kofoid for suggestions and criticisms.

nanas, strawberry jam, molasses, granulated sugar, fresh fish, and meat.

Methods.—For the study of the forms of the organisms found in the different regions of the gut the following method was used. The legs and wings were removed and the specimen was fastened in the dissecting dish with the ventral surface down. A slit was made through the length of the body, extreme care being taken in opening the visceral cavity. After the body wall was pinned back, the digestive tract was carefully removed with dissecting needles, care being taken not to break the intestinal wall. Then the gut was stretched out on a slide in a drop of normal saline solution and covered with a cover slip, so that the exact regions where forms of the parasite occurred could be studied. For rapid examination, however, the head was first cut off, and the gut teased out from the posterior end in normal salt solution.

For permanent mounts the digestive tract was divided into five sections, each section being carefully smeared on a clean slide with the aid of dissecting needles. Then the slide was immediately dipped into a fixative.

In the examination of the larvæ for *Herpetomonas muscarum*, both the posterior and the anterior ends were cut off with sharp scissors, and the digestive tract was teased out and placed on the slide in normal salt solution.

The following fixatives were used: Schaudinn's, with and without acetic acid, Flemming's fluid, Korpenchenko's A and B solutions, and Zenker's, without acetic acid. The first two were used at 60° C. as well as cold. For staining, Delafield's hæmatoxylin, Heidenhain's iron-hæmatoxylin, Mallory's triple stain, and Feulgen's nuclear stain were used with varying degrees of success. Light green and orange-G were used as counterstains. Most satisfactory results in the use of light green were obtained when it was dissolved in 1 per cent solution in clove oil. The flagellates fixed with Schaudinn's solution with acetic acid and stained with Heidenhain's iron-hæmatoxylin gave the best results. Mallory's triple stain did not give good results.

When the organisms were fixed with Korpenchenko's A and B solutions and stained with Heidenhain's iron-hæmatoxylin, very good preparations were obtained, except that the flagella were not shown clearly.

The vital stains used were neutral red and Janus green B in 1 per cent solution in absolute alcohol.

MORPHOLOGY AND DIVISION

The flagellate stage of *Herpetomonas muscarum* is best observed in the crop of the fly. There the flagellates may be found together with the ingested food of the infected fly.

Morphology.—In the crop the writer has seen cysts, and trypaniform, stumpy, and adult forms (Plate 1, figs. 1 to 8; Plate 3, figs. 50 to 53). The trypaniform organism in this region of the gut is, no doubt, the young organism derived from the cyst which has newly been ingested by the fly (Plate 1, figs. 3 to 5). In the midgut have been found long flagellates, stumpy forms, and even encysted forms (Plate 3, figs. 50, 54, 56, and 61). The stumpy forms are numerous in both the posterior and anterior ends of the gut but infrequent in the middle portion. The encysted stage is not very common in this region. The majority of the forms are of the long flagellated and giant types. In the hind gut are found the same forms as in the midgut, but together with many cysts and trypaniform stages. The fæces contain all the forms that are usually found in the hind gut, and are infective to other flies.

In lightly infected flies the Malpighian tubules are not infested with the parasites, but in heavily infected ones the parasites are generally found swarming in the Malpighian tubules. This condition is very evident in the larvæ that are heavily infected with the organisms about twenty-four hours after they are fed with molasses after infection and also in flies that have newly emerged from the pupal stage. This matter will be discussed below. However, once infection is heavy, the parasites are usually found in clusters, either attached to the intestinal wall on the peritrophic membrane or free within the lumen of the intestine. Both on the slides and in living material clusters were observed with the flagella entangled with each other and pointing toward the center. This arrangement also occurs in trypaniform types having short flagella (Plate 2, fig. 21). A number of dividing forms, which the writer believes to occur at all stages of the development of the organism, are usually present.

In the adult stage (Plate 1, figs. 6 and 7) *Herpetomonas muscarum* possesses a long, relatively rigid, slightly flattened body, at the anterior end of which one, or frequently two, long flagella emerge. The length of the organism at this stage ranges from 9 to 36 microns and the width from $1\frac{1}{2}$ to 6 microns. Wenyon (1913) states that the organism is leaflike, resembling a blade

of grass. According to the writer's observations, the flagellates do not resemble a blade of grass, but are round or oval in cross section. A similar form was reported by Becker (1923). The posterior end may be sharply pointed or slightly or broadly rounded.

Although it is possible, as Lwoff and Lwoff (1931) and others have pointed out, that the structure usually called the parabasal body in Trypanosomidæ is not homologous with the parabasal body of Trichomonadidæ, the writer has followed the terminology of recent writers on *Herpetomonas* in this paper.

Anterior to the nucleus is the so-called parabasal body, which varies in size according to the size of the organism, and in form may be ovoid, bar-shaped, heart-shaped, or bean-shaped (Plate 5, fig. 5). In certain stages of the development of the flagellates it is found posterior to the nucleus. Robertson (1927) states that the parabasal body in *Trypanosoma raiæ* is apparently a very dense mass of chromatin, and, colored with Feulgen's nucleal reaction, takes a vivid red stain. The same fact has been observed by the writer in staining *Herpetomonas muscarum* with Feulgen's. Fixed with Schaudinn's solution with acetic acid, or with Korpenchenko's solution, it takes a deep iron-hæmatoxylin stain, and is very well differentiated. In unstained preparations the structure appears as a refractile granule at the base of the axoneme. It is surrounded by a clear space. Wenyon (1913) has stated that this space is inclosed by a membrane, but this has not been verified by the writer.

In *Herpetomonas muscarum* the writer has been unable to find a rhizoplast connecting the nucleus with either the blepharoplast or the parabasal body.

The blepharoplast is located just anterior to the parabasal body (Plate 5, fig. 5, *bleph*) and probably is connected with it by fibrils, although, because of the minute structure of the organism, the writer has not seen these.

According to Strickland (1911) and Wenyon (1913) the clear space anterior to the parabasal body (Plate 5, fig. 5, *ant cl ar*) is the cytostome through which food may be ingested by the flagellate. The writer has studied this structure very carefully, but, like Becker (1923), has been unable to find evidence to support this conclusion.

According to Prowazek (1904) an axial filament in *Herpetomonas muscarum* extends from the blepharoplast with the kinetoplast to another so-called blepharoplast or centrosome, near

the posterior end of the flagellate. With this observation the writer is, for the most part, in agreement.

The rhizostyle commences at the blepharoplast and extends to the posterior end of the cell, terminating in a chromatic granule (Plate 5, fig. 5, *chr gran*). This structure is similar to that described by McCulloch (1915) in *Crithidia leptocoridis*, except that in *Herpetomonas* the rhizostyle is not attached to the nucleus but passes over it (Plate 1, figs. 6 to 14). This condition was clearly ascertained in a few specimens which were stained lightly enough to make clear differentiation of the cellular contents possible.

The writer regards the rhizostyle as a permanent organelle which is present throughout the life history of the flagellate, in the adult and in the trypaniform stages and cysts. In the cyst and trypaniform types the formation of the rhizostyle is similar to that in adults (Plate 2, figs. 25 to 31).

The axoneme of the flagellum (Plate 5, fig. 5, *flag*) arises from the blepharoplast and proceeds anteriorly as a fine line, until it reaches the margin of the cell, where it thickens to form the marginal granule (Plate 5, fig. 5, *mar gran*). The extracytoplasmic portion of the flagellum is much more sturdy and takes a heavier stain than the axoneme. In the adult stage the flagellum is about three times as long as the body.

In fresh material the parasites appear to be possessed of a single flagellum, except those that have evidently reached the later stages of division. But in the fixed material the appearance is different. The organism almost always appears with two flagella lying side by side enclosed by a cytoplasmic sheath (Plate 3, fig. 50).

In organisms that have divided almost completely each individual possesses one flagellum (Plate 2, figs. 17 and 18). The crithidial and trypaniform types in the majority of cases possess only one flagellum, except when the actual division is taking place (Plate 2, fig. 21). The majority of workers, including Patton (1908), Porter (1909), MacKinnon (1910), Becker (1923), and others, have agreed that the biflagellate appearance is due to the tendency of these organisms to be almost constantly in a stage of division.

In the resting stage in the nucleus the karyosome varies considerably in form. The chromatic material of the karyosome may be arranged in four granular masses or may form into a ringlike body with one or more chromatic strands crossing

it (Plate 2, figs. 36 and 40). Forms with a single chromatic mass at the middle of the chromatic ring, and with the chromatic material arranged as in two polar caps, also frequently occur (Plate 2, figs. 41 and 42). The last may be mistaken for a dividing form.

In *Herpetomonas muscarum* a number of globules and the parabasal body may be vitally stained with neutral red. These globules are scattered throughout the body (Plate 4, figs. 66 and 67) although in some cases they are gathered either in the region just posterior to the parabasal body or in the posterior region of the flagellate (Plate 4, fig. 68). The grouping of the neutral red staining globules near the parabasal body makes it difficult to distinguish this body. The writer has also stained the parabasal body of *Trypanosoma lewisi* with neutral red (Plate 4, fig. 69).

By using the method suggested by Hall (1929) the writer obtained the following results with Janus green B. The first structure to take up the dye is the parabasal body which has remarkable affinity and appears as a very bright bluish structure. The other structures that are stained with the dye are the rod-formed mitochondria and vacuoles, scattered throughout the cytoplasm and especially numerous at the anterior and posterior ends of the organism (Plate 4, figs. 62 to 65). One side of the vacuole is always more deeply stained than the other. Shipley (1916) states that some of these vacuoles contain small granules in their anterior part which also stain vitally with Janus green. The writer has searched for these in his preparations, but could not find satisfactory evidence of their presence.

The vacuoles which stain with Janus green are the same in size and form as those which stain with neutral red. Bowen (1928) states that Janus green B will stain the neutral-red-staining vacuoles lightly in time. That the vacuoles that are stained with Janus green are exactly the same in *Herpetomonas muscarum* as those stained with neutral red the writer is, however, unable to affirm at present. When the organisms are stained simultaneously with both dyes, the parabasal body stains dark red, the vacuoles red, and the numerous mitochondria light bluish green.

Binary fission.—The first indication of binary fission is a divided blepharoplast and a duplicated flagellum. According to Patton (1908), Wenyon (1913), MacKinnon (1910), Becker

(1923) and others, the doubling of the flagellum comes about through the growing of another one from the new daughter blepharoplast. The writer has observed the same process of flagellar formation (Plate 1, figs. 6 and 7).

As the blepharoplasts move away from one another, the parabasal body commences at the posterior region to split longitudinally (Plate 1, figs. 8 and 9). With the statement of Becker (1923) that the process of division of the parabasal body is influenced by the blepharoplast, the writer is in full accord. The parabasal body continues to divide, each daughter moving to the periphery of the organism (Plate 1, figs. 9 to 13). They are then separated, one passing to each individual. The parabasal body seems to have an oblique position during binary fission (Plate 1, figs. 11 to 14; Plate 2, figs. 15 to 17), but assumes its position at the same level of the body after the division is completed (Plate 2, fig. 18).

During the splitting of the parabasal body, the division of the nucleus proceeds. The position of the nucleus during elongation is purely accidental (Plate 1, figs. 9 to 14; Plate 3, fig. 52) and can be attributed to the form of the body of the organism.

Just before elongation, fine, scattered chromatin granules, which later form into an equatorial plate, appear at the center. The rest of the chromatic material is gathered into two polar caps with the equatorial plate between them (Plate 2, figs. 44 and 45). This appears to be the metaphase stage. Feebly developed spindle fibers may be observed at this time. This condition may be noted both in the adult and in the encysted form (Plate 2, figs. 26 and 42). The equatorial plate separates into two daughter equatorial plates which move toward the poles (Plate 2, figs. 46 to 48). The number of chromosomes cannot be ascertained. The daughter chromosomes form into groups near the two opposite chromatic caps. Here they become fused with the chromatic caps forming two daughter karyosomes.

During the process of division the body of the cell continually increases in size until the cytoplasm of the mother cell commences to split longitudinally from the anterior to the posterior ends, remaining connected only by a cytoplasmic strand (Plate 2, figs. 15 and 16). As the two daughter individuals grow in size, the cytoplasmic strand breaks, forming two complete individuals with active movements (Plate 2, figs. 17 and 18). In this stage the nucleus assumes the resting condition. The parabasal body divides simultaneously with the dividing nu-

cleus, except in a few cases in which the parabasal body may have already divided before the completion of the nuclear division (Plate 1, fig. 10).

Becker (1923) states that in the trypaniform type usually one flagellum and a nucleus are present, but occasionally dividing forms with two parabasal bodies are found. The writer has confirmed this observation. In addition to this the writer believes that division is similar to that of the adults. The fact that the trypaniform and encysted types divide has not been clearly demonstrated before, so that a few figures have been made to illustrate the stages of division in the cysts found in the hind gut (Plate 2, figs. 25 to 31).

The writer has observed the method of encystment described by Becker (1923) and Strickland (1911), in which the cyst is derived from the trypaniform type. The body of the organism gradually shortens and the middle or posterior portion of the body becomes enlarged. The parabasal body moves backward, taking a position near, just anterior to, or at one side of the nucleus. During this process the flagellum is discarded, leaving the intracystoplasmic axial filament (Plate 2, figs. 24 to 33). The clear space which the writer has named the anterior clear area is conspicuous. The encysted flagellates divide in the same manner as do the adults.

RELATIONSHIP BETWEEN HERPETOMONAS AND FLIES

Relationship to adult flies.—In order to determine the exact mode of infection in the digestive tract of the fly, an experiment was carried on in the following manner.

Eight uninfected flies were placed in each of four clean aquarium jars covered with clean sterilized cheesecloth, and fed with newly infested meat from the cage where wild flies had fed on it for two hours. After twenty-four hours when aquarium jar 1 was examined, five of the flies were infected with *Herpetomonas*. The infection occurred in the crop and the midintestine. On the third day the same number were infected in aquarium jar 2. The infection was much heavier and occurred in the crop, midgut and anterior end of the hind gut. Rounded forms were not found. On the sixth day the infection was much heavier in the flies in aquarium jar 3. Stumpy as well as a large number of flagellated forms were found throughout the digestive tract. The flies in aquarium 4 were left undisturbed until the sixteenth day, so that the infection with flagellates became very heavy. The intestine swarmed

with flagellates of all forms. Stumpy forms occurred in the crop, rarely in the midintestine, and in the posterior part of the midgut and the anterior portion of the hind gut. Flagellated and round stumpy forms were found in the crop, flagellated trypaniform stages and cysts in the midintestine, flagellated forms, large trypaniform, and encysted stages in enormous numbers in the hind gut.

This experiment demonstrates the manner in which the infection is carried from fly to fly by means of contaminated food.

In an experiment to determine the effect, if any, of the parasites upon the host, two cages were used, one having wild and the other uninfected flies. The flies in the cages were fed with uninfected fresh meat. Beginning two hours later, the flies were left without food for more than twenty-four hours. Both infected and uninfected flies were examined for food content. The infected flies had no food left. Some of them were dying, whereas the clean ones had food left and were still active.

This experiment confirms Patton's (1910) conclusion that the swarm of flagellates passes up to where the food is and absorbs it. The same author stated that when infected flies were fed with jam and juice of horse dung the parasites completely disappeared. The writer fed flies with strawberry jam alone and this did not affect the growth of the parasites.

Relationship to fly larvæ.—A diversity of opinion has existed concerning the relation of *Herpetomonas* to fly larvæ. According to Prowazek (1904), transmission of *Herpetomonas muscarum* is hereditary, by which he means that the parasites are passed through various stages of development of the fly from the egg to the adult. Patton (1910), in his experimental infection of *Musca nebulo,* found no evidence of hereditary transmission. He further stated that in his investigation the parasites were not found in the larvæ bred in horse dung, and that the long flagellates were found in very young larvæ fed on the dead bodies of flies, but never in the mature larvæ, pupæ, or flies bred from these larvæ. The same author, however, reported in 1921 that the larvæ of *Musca nebulo* are commonly infected with *Herpetomonas muscarum,* and that the infection is carried over to the adults, so that when the adults hatch out they are infected. Becker (1923) gives evidence that the organism is not transmitted hereditarily and cannot be found in the digestive tract of the larvæ. He wrote, "During the months of July and August, 1922, I examined two hundred fly larvæ, principally *Lucilia* and *Phormia* in various stages of development, taken

from the most exposed places in Westport dump, Baltimore. No infected larvæ could be found. I also examined sixty *Musca domestica* larvæ taken from a pile of horse manure but I found no infection." Strickland (1911) was unable to find infected larvæ in *Lucilia.* Prowazek (1904) found infected *Sarcophaga* larvæ. MacKinnon (1910) reported larval infection in *Scatophaga* and *Homalomyia,* but was unable to find it in larvæ of *Musca domestica.* Becker states, "It is difficult to draw any conclusion from the conflicting observations of various authors, but it seems that one or two factors must be at work here. It is possible that the larvæ of certain species of muscoid flies are capable of infection with *Herpetomonas,* and that certain others are not. Then there is another possibility that certain environmental conditions, relating to moisture, temperature, character of food, flora of the larval intestine, etc., are factors which determine whether or not the larvæ could be infected."

The writer undertook investigations to determine whether the larvæ of flies are infected with the parasites. The back of a restaurant was selected as a site to breed fly larvæ. The restaurant had a large-sized fly trap beneath which heads of chicken and fresh fish were placed for flies to feed on. After feeding they flew upward and were caught in the trap. Before the female flies left the baits, they laid their eggs on them. Within twenty-four hours, as the weather at that time was favorable for hatching, the eggs hatched. The larvæ fed on the chicken heads and fish. The baits were constantly infected with the flagellates by flies laying eggs and feeding on them. More than sixty larvæ, both young and old, were examined but not a single one was found infected.

Another experiment was performed to determine whether the larvæ of *Lucilia sericata* could be naturally infected by *Herpetomonas muscarum.*

More than fifty infected flies were put in a cage. The flies were fed with fresh beef liver placed in a petri dish. The liver was kept moist with sterile normal saline solution until 10 P. M. That same morning a large number of eggs previously laid were placed on the liver. At 10 A. M. the next morning the eggs were already hatched. Eight larvæ were examined with negative results. In the evening six of them were examined, and found uninfected. The larvæ and the infected liver were transferred into three test tubes. All the test tubes were placed in aquarium jars with sterile sand. The jars were covered with

cheesecloth and put in a large box covered also with cheesecloth. Every morning some of them were examined, and no infection was found. After the larvæ stopped feeding, they crawled out of the test tubes, dropping on the sand, into which they burrowed to pupate. In two weeks they emerged from the pupæ as adults. The newly emerged flies were examined with no positive results.

In this case the larvæ feeding on infective meat were not infected. The results of this experiment did not agree with those reported by Patton (1921).

The writer performed another experiment to determine whether the larvæ of *Lucilia* could be induced to take in *Herpetomonas* by feeding on dead flies.

Wild flies were dissected and put into a test tube, where they were kept moist by the addition of a few drops of sterile normal saline solution. Twenty-one one-day-old larvæ were dropped into the test tube. After six hours one of them was examined and found to contain herpetomonads. The next day three others were found infected. Fresh dead flies were added to maintain a sufficient food supply.

Eight of the larvæ were taken out and fed on old meat in another test tube. Twenty-four hours later no infection was found. In this case the old meat used by the larvæ was not favorable for the growth of the parasites.

Two and three days after the beginning of the experiment the rest of the larvæ feeding on dead flies were examined and found infected.

As the larvæ could be infected with the parasites by feeding on the infective digestive tracts of the flies moistened with normal saline solution, the writer carried on further experiments to determine whether or not the larvæ can be infected with the parasites at any age and the infection carried over the adult stage under natural conditions. Horse meat, yolk of egg, and molasses were selected as food for the larvæ during the experiment, and they were kept at room temperature.

In the first of these experiments fifty two-hour larvæ were fed with heavily infected digestive tracts of the flies moistened with normal saline solution. A day later eight were examined, and six found infected. The contents of the digestive tract included an enormous number of bacteria and flagellates of different forms. The larvæ were transferred to fresh horse meat in a test tube. A day later, of eight examined, four were positive. Adults and a few round forms were present. The bacterial infection was very heavy, and the flagellate infection light.

A day later, of six examined, all were negative. On the next day the larvæ migrated to sawdust to pupate. Eight were examined and all found negative. Five flies which emerged twelve days later were negative.

In a second experiment fifty twenty-four-hour-old larvæ were infected with the parasites in the same way. The next day eight were examined and all found positive. The infection was light in some, heavy in others. The larvæ were transferred to fresh horse meat in a test tube. A day later, of ten examined, eight were positive. The infection, which was light, included round and trypaniform forms. The bacterial infection was very heavy. On the next day, of eight examined, seven were negative and one was lightly infected. The rest migrated for pupation. Two weeks later the flies began to emerge. All were negative for *Herpetomonas*, but the bacterial contents of the digestive tracts were heavy.

In a third experiment fifty forty-eight-hour-old larvæ were fed with infective digestive tracts of flies moistened with normal saline solution. A day later, of ten examined, all were positive. The remainder were transferred to fresh horse meat in a test tube. The next day only five of ten were positive, and these contained rounded and trypaniform stages. The same day at night the larvæ began to migrate to pupate. When two weeks later the adults emerged, all were found negative.

In another experiment larvæ approximately three days old were fed with digestive tracts of flies heavily infected with flagellates. Larvæ of this age are somewhat difficult to infect, as the sudden change of nutrition seems to hinder them from taking the new food, so that a method was devised that would force them to emerge into the infective food. Furthermore, at this age, when they had been continually feeding on the ground horse meat, it is nearly time for them to stop feeding.

Twenty-five three-day-old larvæ were dropped into the infective medium in a vial, and a test tube inserted into it leaving an inch space at the bottom where the larvæ were forced to stay, with an air space between the outside of the test tube and the inside of the vial. This method is remarkably effective in the infection of the larvæ as they are forced to emerge into the infective food and thus to ingest the parasites in the medium.

A day later six were examined, and four were infected. They were left free to migrate into the sterile sawdust in a glass jar covered with perforated tin cans.

Fifteen days later the flies began to emerge, and were examined as they gradually emerged. None was found infected with *Herpetomonas.*

Further experiments were carried on with ground fresh horse meat, yolk of egg, and molasses (New Orleans molasses) as food for the larvæ.

In the first of these, fifty twenty-four-hour-old larvæ were infected with *Herpetomonas* by feeding on heavily infected digestive tracts of wild flies. They did not grow much larger than they had been, owing to lack of food. After infection they were transferred to the fresh horse meat. They grew very fast and the flagellates were beginning to disintegrate when they were examined the next day. They were again transferred to the yolk of egg. Within twenty-four hours they were carefully examined for parasites. A light infection was found. The larvæ did not grow as rapidly as when they were fed with meat.

They were at last transferred to molasses in another test tube. After twenty-four hours they were examined again for parasites. Strange as it may seem, the infection was found very heavy; even the Malpighian tubules were swarming with flagellates. The larvæ did not grow in size and the length of the time to pupation was increased to eight days. In spite of their undersized condition they pupated and emerged in less than the usual time. The emerged flies were carefully examined for flagellates, with the result that ten out of fifteen were found heavily infected both in the guts and in the Malpighian tubules.

In a second experiment a mixture of the three kinds of food was fed to the infected twenty-four-hour-old larvæ. They grew faster and became as large as those which had been fed with meat alone. The flies took approximately fifteen days to emerge and were examined at the time they appeared. Two out of twenty-five examined were heavily infected.

The results of these experiments indicate that the larvæ of different ages can be infected with the organisms either heavily or lightly, but the flagellates disintegrate from twenty-four to forty-eight hours after the infection has taken place by rounding up or dying out when the larvæ are fed with horse meat alone. Undoubtedly, the old meat does not favor the growth of the parasites because of the bacterial and perhaps other elements in it that do not give them favorable nutritive value. Entirely different results have been obtained by feeding the

larvæ on various diets; namely, fresh horse meat, yolk of egg, and molasses. The parasites grew rapidly and were carried to the pupal and adult stages.

Hereditary transmission of infection.—An experiment was then carried on to determine whether transmission of *Herpetomonas muscarum* is hereditary. The materials used were a large box covered with cheesecloth, a tin can, fish, sand, infected flies, and fly eggs.

Wild flies were put in a cage and fed with fresh fish. By watching them and using a cage appropriate for the work, the writer was able to catch the exact individuals that laid the eggs on the food, and these were examined for parasites and found to be heavily infected. The newly laid eggs were transferred to the sterile fish which were placed in a shallow sterile tin can in a sterile aquarium jar covered with cheesecloth. In twenty-four hours the eggs were hatched. Within five days the larvæ stopped feeding; they migrated into the sand under the tin can to pupate. Two weeks later adult flies emerged from the pupal stage. These newly produced individuals were examined for *Herpetomonas* and found uninfected. The experiment was carried to the third generation with the same result.

This experiment showed that the flagellates were not hereditarily transmitted.

DISCUSSION

Why the larvæ generally are not infected when they are gathered in natural conditions can be understood by the kinds of food they feed on, their behavior during feeding, and their reaction to some environmental factors. They are usually found feeding on carrion, manure, garbage, and other refuse. These kinds of food are not very well suited to the livelihood of parasites, owing to their bacterial content and other elements which may become detrimental to growth of the flagellates unless favorable food for the organisms is ingested. Food that really favors the growth of parasites is not usually used by larvæ, as they are generally limited to the same food from the time they have started feeding to the moment they are ready to pupate. In this way there are no cycles of constant food changes, such as the adult flies generally have. Owing to the presence of toothlike structures, which are used for grabbing rather than for sucking, the larvæ are different in food habits and method of taking in food from the adult flies, to which the lack of fluid to thin out the bacterial contents for organisms to freely

move around can be attributed. When the bacterial contents are thick the parasites lose their power of motion, resulting in their dying out or rounding up. Patton (1921) has stated that overgrown bacteria in culture cause the death and the rounding up of the parasites.

Another factor, it seems to the writer, is the method of feeding. Observation shows that the larvæ are not generally found on the surface but are partially buried in the food with their posterior ends out. This position often occurs after the surface of the food, for example, carrion, has been used up, leaving already used materials at the top and unused food at the bottom on which the animals feed. Undoubtedly the waste materials at the top are not being used, and are not suited to the existence of the parasites in case contamination by adult flies occurs.

Experimentally, the larvæ are negative to light. If they happen to be in the light after hatching they immediately migrate to the dark or dig themselves into the food, which keeps them free from infection of the flagellates from the newly extruded excrement of flies.

In adult flies constant changes of food content take place in the alimentary canal. The writer has observed constantly during the time he was catching his specimens that flies feed on various kinds of food. They may be found on flowers; in decayed or fresh fruits, such as apples, bananas, grapes, and others; on both spoiled and fresh meat by which they are generally attracted to lay their eggs; and in decayed vegetables, manure, sugary food, and filthy materials. The chances of infection are very great, owing to the ability of flies to move constantly from food to food. The larvæ have little or no chance for infection. Their habits, their reaction to environment, and the kind of nutrition they consequently get, militate against the presence of the flagellates in the larvæ.

SUMMARY

1. The flagellate *Herpetomonas muscarum* is entozoic in *Lucilia sericata* in Berkeley, California. About 99 per cent of the flies are found infected during fall and spring, but seldom during winter, from the latter part of December to the latter part of February.

2. The infection occurs throughout the alimentary canal and, if it is heavy, even in the Malpighian tubules.

3. A study has been made of the morphology of the flagellates.

4. The structures that can be stained intravitally with Janus green are the parabasal body, mitochondria and vacuoles; and with neutral red the vacuoles and the parabasal body.

5. The cysts and trypaniform types divide by the same process as that taking place in the adults, except that nuclei do not exhibit various forms in the resting stage.

6. The flies and larvæ may be infected by any type of flagellate.

7. The larvæ can be infected at any age during the larval stage by feeding on infective digestive tracts of the wild flies. As the flagellates live only from twenty-four to forty-eight hours when the larvæ are fed with horse meat, it cannot be expected that the infection could be carried over to the imago.

8. The parasites are carried over to the adult flies from the time the larvæ are infected, when the larvæ are fed with molasses, or with a mixture of 1 part molasses, 2 parts yolk of egg, and 10 parts of horse meat.

9. The normal absence of the flagellates in the alimentary canals of the larvæ is due to the kind of food.

10. No hereditary transmission of the parasites occurs in the fly.

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ILLUSTRATIONS

[All figures in Plates 1 to 3 were made from specimens fixed with Schaudinns' fluid and stained with Heidenhains' iron-hæmatoxylin; those in Plate 4 were made from living material stained with Janus green B and neutral red; Plate 5, figs. 1 to 4 drawn from fixed materials; fig. 5, a diagrammatic drawing showing structures.]

PLATE 1

FIGS. 1 and 2. Cysts newly ingested by a fly, showing rhizostyles and chromatic granules; × 1,466.

3 to 5. Individuals with their axonemes grown out of the cells as flagella, showing the gradual migration of the parabasal body to the anterior and that of the nuclei to the middle of the cells. The rhizostyles also elongate simultaneously with the body of the cells; × 1,466.

6 and 7. Individuals in resting stages, showing the gradual growth of the rhizostyles to join the daughter chromatic granules. The rhizostyle is passing over the nucleus; × 1,466.

FIG. 8. The nucleus is in the prophase stage with the chromomeres at the middle of the chromatic ring; × 1,466.

FIGS. 9 and 10. Flagellates with their nuclei in the metaphase stage, each having two polar chromatic caps and the equatorial plate between them. The positions of the elongation of the nuclei represent two types, oblique and longitudinal. The parabasal bodies are dividing; × 1,466.

11 and 12. The nucleus of fig. 11 is in the early anaphase stage, and that of fig. 12 in the late anaphase, both with the persistent nuclear membrane around them; × 1,466.

13 and 14. In fig. 13 the two daughter nuclei are connected dumb-bell fashion. The parabasal body has almost completely divided. In fig. 14 the achromatic mass has been cut in by the nuclear membrane, thus forming two completely separated nuclei; × 1,466.

PLATE 2

FIG. 15. Thin cytoplasmic sheath at the middle of the cell; × 1,466.

FIGS. 16 and 17. Gradual splitting of the cell body into two daughter individuals, each having a rhizostyle; × 1,466.

FIG. 18. A completely divided individual, with the blepharoplast still undivided showing a rhizostyle and an axoneme. It represents the stage just before the trypanosome type; × 1,466.

19. Trypanosome type with parabasal migrating toward the region posterior to the nucleus; × 1,466.

20. An individual with the parabasal body at the posterior region of the cell; × 1,466.

21. Trypaniform flagellates in rosette formation; × 1,466.

22. A crithidial form. Note the undulating membrane; × 1,466.

FIG. 23. A trypaniform flagellate which has almost completely rounded and formed into a cyst; × 1,466.

24. Cyst with distinct cyst wall and resting nucleus; × 1,466.

25. Cyst with its parabasal body located anteriorly; × 1,466.

FIGS. 26 to 28. Cysts with their parabasal bodies and nuclei in the process of division; × 1,466.

29 to 31. Cysts with their nuclei and parabasal bodies completely divided; × 1,466.

32 and 33. The two completely divided cysts; × 1,466.

34 and 49. Diagrammatic representation of the different nuclear stages; × 1,466.

34 to 42. Various forms of nuclei in resting stages, from the adult long-flagellate forms; × 1,466.

FIG. 43. Nucleus in the prophase stage with the chromatic granules (chromomeres) in the chromatic ring; × 1,466.

FIGS. 44 to 48. Nuclei showing stages of mitotic division, from the early metaphase to the late anaphase; × 1,466.

FIG. 49. Nucleus in the late telophase showing achromatic mass connecting the two daughter nuclei; × 1,466.

PLATE 3

FIG. 50. A stumpy type with the nucleus in the resting stage, showing the incomplete growing flagellum and rhizostyle; × 1,466.

FIGS. 51 and 52. Individuals with nuclei dividing horizontally to the body. In fig. 51 the rhizostyle is clearly shown, originating from the blepharoplast; × 1,466.

FIG. 53. The completely divided stumpy type; × 1,466.

54. A giant-type organism with a nucleus in metaphase stage; × 1,466.

55. A clublike form with the posterior end enlarged; × 1,466.

FIGS. 56 to 58. Gradual shortening of the body; × 1,466.

59 and 60. The individuals have already rounded up, with their long flagella still connected with the axoneme. The rhizostyle also persists; × 1,466.

FIG. 61. The flagella have already fallen off, leaving only the rudimentary axonemes; × 1,466.

PLATE 4

FIGS. 62 to 65. Aggregation of mitochondria in both anterior and posterior portions of the cell. Stained with Janus green B; × 1,980.

66 to 68. Grouping of the vacuoles (vacuome) in posterior and anterior regions. Stained with neutral red. Both stains stain the parabasal body; × 1,980.

FIG. 69. *Trypanosoma lewisi* with its parabasal body stained with neutral red.

PLATE 5

FIG. 1. *Herpetomonas muscarum* (Leidy), undivided trypaniform type; × 2,000.

2. Dividing trypaniform form; × 2,000.

FIG. 3. Cyst with nucleus in resting stage; × 2,000.
4. Dividing cyst; × 2,000.
5. *Herpetomonas muscarum* (Leidy), a diagrammatic drawing showing structures. *flag*, Flagellum; *mar gran*, marginal granule; *ant cl ar*, anterior clear area; *bleph*, blepharoplast; *pb*, parabasal body; *rhiz*, rhizostyle; *perip*, periplast; *nuc mem*, nuclear membrane; *kar*, karyosome; *chr*, "chromidia;" *chr gran*, chromatic granule.

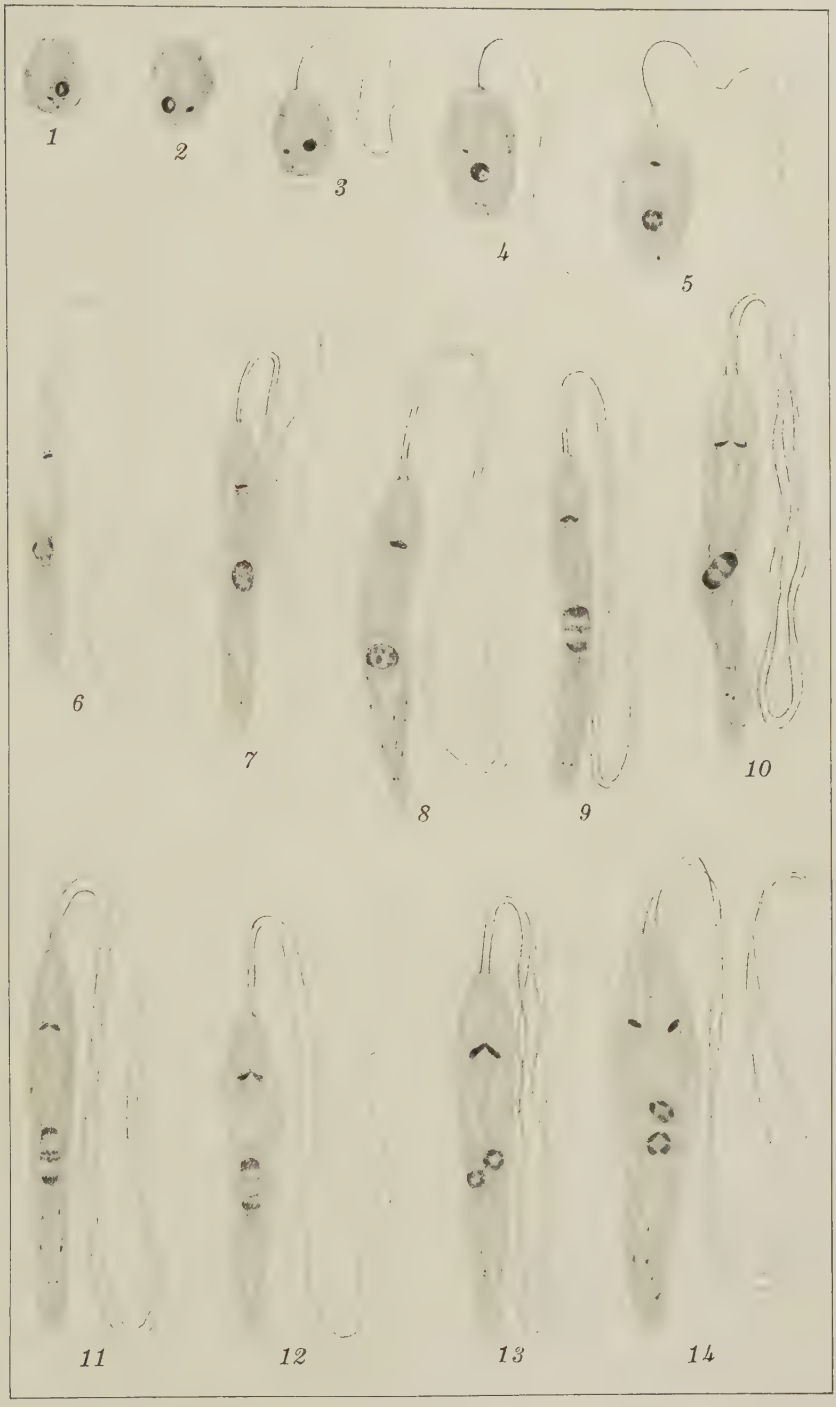

PLATE 1.

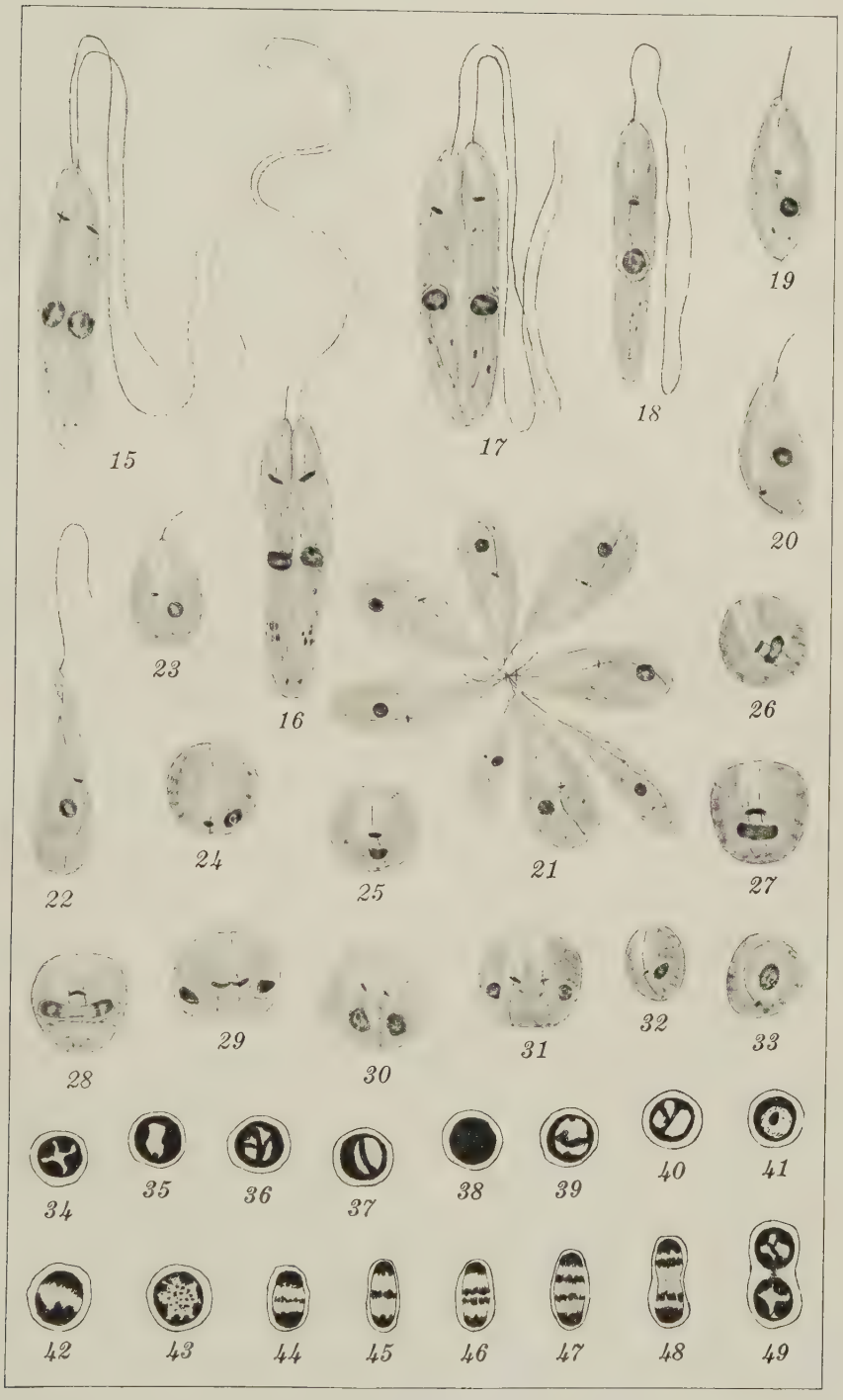
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PLATE 2.

PLATE 3.

PLATE 4.

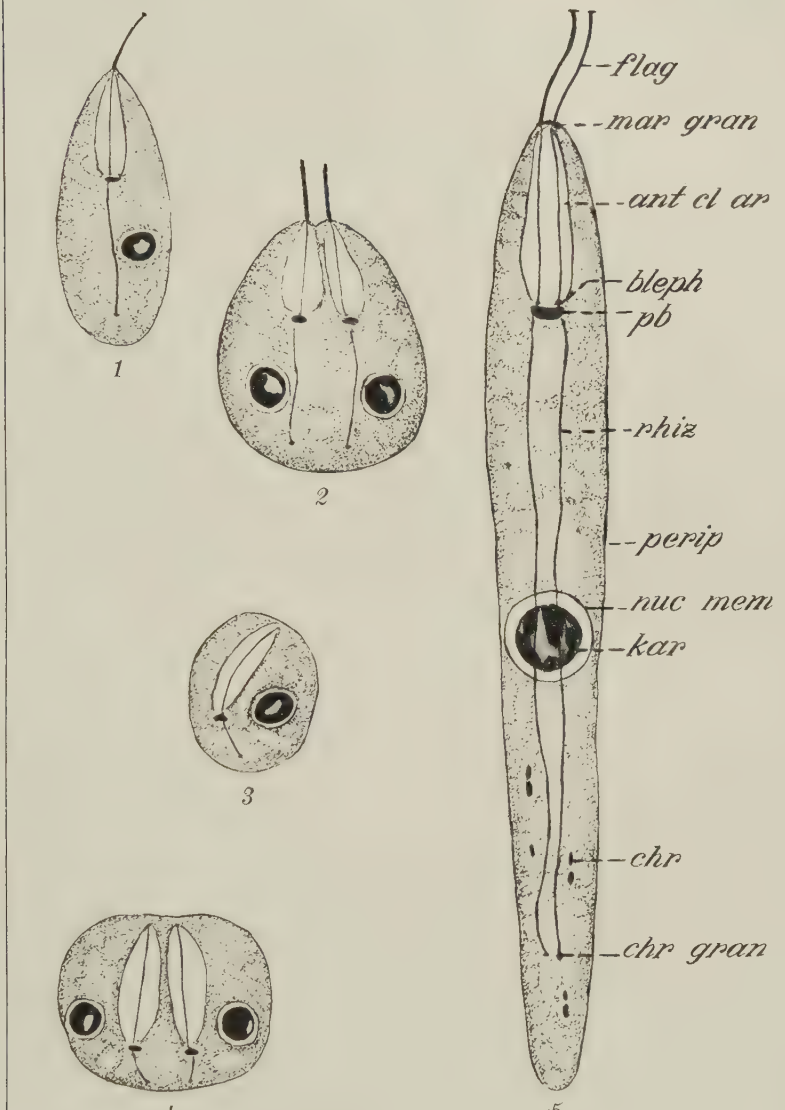
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PLATE 5.

THE BIOLOGY OF MOINA MACROCOPA STRAUS WITH SPECIAL REFERENCE TO ARTIFICIAL CULTURE [1]

By GERVASIO C. BELLOSILLO

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ONE PLATE

INTRODUCTION

Moina macrocopa Straus, a species of Cladocera, is found in various temporary pools in Manila and its immediate vicinity. This organism is very prolific, though relatively short-lived. It is considered by almost all aquarists and fish lovers as the best live food for nearly all ornamental fishes, both imported and native. As such it is nutritive and palatable, promotes early fecundity, and can be fed to any size of fish except the newly born, which feed on microörganisms.

SYSTEMATIC POSITION AND SYNONOMY

Dr. Robert Gurney,[2] tentatively identified our waterflea as *Moina dubia* Richard, but sent the specimen to Mr. D. J. Scourfield [3] for verification. The latter regarded it as not of that species, and placed it as *Moina* sp.

Except in the coloration, which is influenced by food, our material fully answers Birge's (1918) description of *Moina macrocopa* Straus:

> No supra-ocular depression; claws not pectinate; antennules of male with sense seta in middle or below; first foot of male with long flagellum. *Moina macrocopa* Straus 1820.
>
> Not very transparent; yellowish or greenish. Head extended. Terminal part of post-abdomen long, with 10-12 spines besides bident. Two ephippial eggs. ♂ with elongated head; 5-6 hooks on antennule. Length, ♀ to 1.8 mm.; ♂, 0.5-0.6 mm.
>
> Pools, Wisconsin, Nebraska, Colorado, N. Dakota; doubtless widely distributed.

According to Birge (1918) the genus *Daphnia* was first established by Muller (1785). Straus in 1820 described a new

[1] Read before the Fourth Philippine Science Convention, February 24, 1937.

[2] Bayworth Corner, Boar Hill, Oxford, England.

[3] 6 Chadwick Road, Leytonstone, England.

species, *Daphnia macrocopus*. Baird in 1850 founded the genus *Moina* and described a species, *M. rectirostris*, in which he included *D. macrocopus* Straus. Herrick (1895) believed *D. macrocopus* to be synonymous with *M. brachiata* Jurine. Hudendorff in 1878 reported *M. flagellata* from Central Russia which Herrick (1895) regarded as the same species as *M. paradoxa* Weismann, believed by him to be identical to *M. fisherii* Hellich and *M. azorica* Moneiz. Sars (1903) described *M. macrocopa* Straus from Central Asia. Birge in 1918 considered *M. flagellata* Hudendorff and *M. paradoxa* to be the same as *M. macrocopa*, and regarded *M. brachiata* and *M. rectirostris* as distinct species.

GEOGRAPHICAL DISTRIBUTION

This species is widely distributed throughout the world. Straus (1820), Weismann (1877), and Hudendorff (1776) reported it from Europe, Sars (1803) from Central Asia and (1916) South Africa, Herrick (1895) and Birge (1918) from the United States, Bar (1924) from Ceylon and South America, and Terao and Tanaka (1928) from Japan.

In Manila, in San Pedro Makati and Pasay, Rizal Province, and in Calamba, Laguna Province, it is found in practically all temporary standing fresh water, near houses. It is probably well distributed throughout the Philippines, although its distribution there has not yet been studied.

HISTORICAL REVIEW

In 1879 Weismann, according to Banta (1925), set forth the hypothesis that in Cladocera there is an innate sexual cycle quite independent of environmental influences. Grosvenor and Smith (1913), working on *Moina rectirostris*, found that the innate sexual manifestations in Cladocera are not influenced by internal factors but by environmental influences. In 1925 Banta concurred in this view with regard to *M. macrocopa*.

Allen and Banta (1929) pointed out that the fertilized sexual egg of *M. macrocopa* hatches out as a female, which reproduces parthenogenetically young that may be either parthenogenetic females, sexual females, or males. The parthenogenetic females of this and succeeding generations may also reproduce all three kinds of individuals. Similar sexual differentiation occurs in *M. rectirostris*, as found by Grosvenor and Smith (1913).

Banta (1921) states that bacteria constitute the principal food element of soil-manure cultures. He further states that "while a certain amount of uniformity is attainable in such a

culture medium such mass cultures are quite variable and it is obvious that this can scarcely be considered a "Standard" food. It is probable that the proper bacteria could be reared on agar plates at definite intervals and a really standard food thus obtained." Stuart and Banta (1931) announced that the number of bacteria available as food for Cladocera in different make-ups and dilutions of manure solution used in culturing these animals are found by counts to be exceedingly variable.

Viehoever (1933) recommends yeast as a remarkable food concentrate, partially replacing meat, having three times the nutrient of beef, and serving to overcome weakness and lack of appetite in diabetic patients, possibly on account of its high (2 per cent) content of lecithin or nucleic substance, and facilitating the breeding of *Daphnia*. Viosca, according to him, provides infusoria as food for *Daphnia* once or twice a day and feed his infusoria with a bacterial culture about every second day (1 glass per crock or tub). He further states that cotton-seed meal in amounts of 0.01 to 0.02 per cent is very good for laboratory breeding, and that excellent results are obtained with soy-bean-flour experiments in maximum amounts of 0.01 to 0.02 per cent.

As a *Moina* culture medium, Terao and Tanaka (1928) used one part of Jenning's and Lynch's solution of oatmeal for rearing Rotifera, and two parts of rain water which contained a large quantity of *Scendesmus*.

No literature is available on postembryonic development, the number of fecundities and the number of young produced during a life span, the hydrogen-ion concentration range, and the rôle of iron in culture media of *Moina macrocopa*.

Although apparently long present in the Philippines, the organism was not known there until early in 1934, when ornamental fish culture was in its infancy in the Islands. Aquarists who first found it by accident mistook it for *Daphnia* sp.

COMMON NAMES

Daphnia are commonly known as waterfleas. Our species is at present generally called "daphnia" in Manila. Like *Moina*, *Daphnia* is a genus belonging to the family Daphnidæ, order Cladocera, and is at present not found in the Philippines. Since daphnia is used as a common name for a different genus, "moina," being the right name, should be used as the common name of this waterflea. The native name applied to this animal is "kutung tubig." Unfortunately, it is little used.

OBJECT OF THE PRESENT WORK

Due to the rapid growth of ornamental fish culture in Manila and in other parts of the Philippines, the economic importance of moina for fish food becomes more and more apparent. Since this animal is abundant only during the rainy season, there is great need of raising it artificially. Before that can be done, however, considerable biological investigation is necessary with regard to the life history, and the invironmental requirements of this organism.

The present work was undertaken in answer to this need. Field observations were made in Manila and Pasay, Rizal Province, at various suitable times in order to determine seasonal occurrence and abundance of natural enemies, types of natural habitat, and other biological data. Observations were made early in the morning, at noon, in the afternoon, and shortly after rainy weather.

DESCRIPTIONS

Parthenogenetic female (*Plate 1, figs. 6 and 7*).—Adult, 0.9 to 1.8 millimeters long, yellowish to reddish. Body thick and heavy, not completely covered by carapace. Carapace without spine. Carapace valves obscurely reticulated, subrounded-oval when seen laterally, ventral margins armed with setæ. Antennule small and hairy, slightly tapering on both ends, one-jointed, freely movable, with terminal sensory filaments, and a seta almost at middle. Head extended, large, thick, rounded in front and without supra-ocular depression. Abdomen represented by a horseshoe-shaped fold. Postabdomen extended into a conical postanal part, bearing from eight to ten ciliated spines and a bident, and dorsally pubescent with rows of hairs. Claws small, each with a row of fine hairs at both lateral aspects. Abdominal setæ very long, two-jointed, first bare, second ciliated. Antennæ strong, beset with rows of hairs, setæ of rami ciliated. Dorsal ramus four-jointed, with a spine and three two-jointed setæ at distal end of last segment, one at distal end of third segment, and a spine attached dorsally at end of second segment. Ventral ramus three-jointed with three two-jointed setæ at distal end of the third and one each at distal end of the first and second segments. At distal end of the main trunk with a two-jointed long seta between the rami, and two two-jointed long setæ lying side by side near the basal region. Mature parthenogenetic female almost monstrously deformed by

the young in the brood sac, the latter cavity subspherical in shape, extending laterally beyond the ordinary limit of the shell (Plate 1, fig. 7).

Sexual female.—Adult essentially like the parthenogenetic female. Sexual female more normal in shape. Dorsal half of valves mostly transformed by very pronounced reticular markings and thickening into a two-valve padlike case for the two ova. It can be easily distinguished through the naked eye by the opaque appearance of the ephippium (Plate 1, fig. 5) at the back of the animal.

Male.—Adult elongated, much smaller than female, 0.5 to 0.7 millimeter in length. Head elongated and comparatively large, less strongly arched above, and much produced forward. Eye comparatively large, with about eight lenses. Antennules curved, with two sensory setæ in the middle, one longer than the other, and at the end five to six claws and sensory filaments. First foot with a long hairy three-jointed flagellum, two two-jointed ciliated setæ, and two spines, one seta moderately short and the other long. Carapace comparatively long, not completely covering the body. Carapace valves, when seen laterally, subrounded oval, ventral margins bearing somewhat distant setæ.

Female nymphal instars.—Tables 1 and 2 show three nymphal instars credited to this species. First instar 0.40 to 0.65 millimeter long, hyaline. Second instar 0.68 to 0.90 millimeter long, slightly yellowish. The nymph gradually increases in size until it reaches a length of about 0.95 millimeter before the third molting. General characteristics similar to those of adults.

Eggs.—This organism, as nearly all Cladocera, has two types of eggs, the parthenogenetic and the sexual. When the eggs are ripe they differ markedly in appearance.

Parthenogenetic eggs newly laid in the egg pouch are oblong and yellowish. They gradually become rounded and large, reaching a length of about 0.13 millimeter. Parthenogenetic eggs produce both males and females (Plate 1, fig. 6).

Two sexual eggs are inclosed in an ephippial case. They are rounded-oblong, and can stand fairly hard pressure. They are about 0.18 millimeter wide and 0.20 millimeter long. The ephippium which envelops the sexual egg is bean-shaped, apparently inflated, anteriorly broader than posteriorly, dorsally thickened, flattened above, and nearly straight when viewed laterally, and ventrally thin, subrounded, and hyaline. Sexual eggs produce only females.

LIFE HISTORY AND HABITS

The life-history records in the present report are based on observations under natural conditions and on cultures carried under laboratory conditions.

Unless otherwise stated, the materials used were drawn mainly from one parthenogenetic female obtained in August, 1934, from a pool near Balagtas Street, Pasay, Rizal Province, and cultured in soil-manure medium.

In the study of the type of sexes the ephippial eggs were gathered and placed on pieces of blotting paper in a petri dish by means of a pipette, and dried either in the sunshine or in an insect drier; then they were transferred to the soil-manure medium to hatch. The newly hatched young were either isolated singly or in groups. In the study of the life span and the proportion of sexes under uncrowded conditions, sister moina were singly isolated in 1- × 3-inch vials, each containing 20 cc of soil-mungo or soil-manure medium. Examination of the various stages was made at intervals of from ½ hour to 1 hour, or oftener, by placing the vials one at a time before a bright diffused light during the day and before an electric light at night, for purposes of observing the process of molting and noting the already dropped exuviæ. To study the proportion of sexes under crowded conditions, ten mothers were placed together in bottles each containing 100 cc of soil-manure medium, and the medium was changed daily. For purposes of counting, the animals were poured into a cone-shaped strainer made of fine silk cloth, drawn out of the strainer by means of a pipette, and placed in watch glasses containing small amounts of water to which a little of 1 per cent chloritone had been added. The mothers were selected from the groups and returned to their respective bottles. The counting was greatly facilitated by the use of a low-power Zeiss dissecting binocular microscope.

PARTHENOGENETIC FEMALE

Postembryonic period.—In Tables 1 and 2 the period between molts varies from 6.08 to 11 hours in individuals cultured in soil-mungo medium, and from 6.5 to 10 hours in those cultured in soil-manure. All of the thirty-two singly isolated organisms cast their skins three times during their postembryonic development. In those cultured in soil-mungo medium, the average duration of the first stage is 6.73 hours, of the second, 8.56, and of the third, 9.67, with 24.89 hours as the total average period

from birth to the adult stage. In those cultured in soil-manure, the average duration of the first stage is 6.53 hours, of the second, 8, and of the third, 9.73; with the total average period of 24.26 hours between the time of birth to that of adulthood.

Life span.—Terao and Tanaka (1930) found that *M. macrocopa* cultured in constant temperature has an average duration of life of 4.778 days in 35° C., 6.515 in 27° C., 9.283 in 20° C., 14.365 in 15° C., 10.188 in 9° C., and 4.158 in 4° C. The maximum duration of life in moina is then at 15° C. Tables 1 and 2 give the life span of moina at room temperature, ranging between 27° C. and 31° C. in Table 1 and between 26 and 31.5 in Table 2.

Tables 1 and 2 show that 100 per cent went through their postembryonic development. The span of life in Table 1 varies greatly between the minimum duration of 4.23 days and the maximum of 13.50 days, the average being 8.72 days. In Table 2 the minimum is 7.96 days, and the maximum 14.15 days, the average being 12.82 days. The average life span in soil-manure (Table 2) is proportionally higher than that of Terao and Tanaka at nearly the same temperature; the difference may be due to food conditions.

Fecundity.—The total number of young produced during a life span by each of the thirty-two cultures, together with the total number of young in each brood and the average number per brood, is given in Tables 3 and 4. The number of young produced varied from 7 to 24 in Table 3, and from 3 to 32 in Table 4, showing exceedingly great difference in the production of young. Large numbers of young were produced in the second, third, fourth, and fifth broods, averaging between 12.26 and 18.8 in soil-mungo medium and between 16.23 and 28.38 in soil-manure medium. In soil-mungo medium the minimum number of young produced per life span, where the mother dies after the third brood, is 65, and the maximum, where the mother dies after the eleventh brood, is 165; only two mothers out of nineteen lived beyond the eleventh brood. In soil-manure medium the minimum number of young produced, where the mother dies after the seventh brood, is 126, and the maximum, where the mother dies after the thirteenth brood, is 214; three mothers out of thirteen lived beyond the thirteenth brood. The fecundity of the thirteen mothers that produced 2,846 young in soil-horse-manure medium is considerably higher than that of the nineteen mothers that produced 2,091 young in mungo-culture medium.

TABLE 1.—*Life span, interval between birth and adulthood, and length of reproductive period in soil-mungo medium.*

Culture No.	Duration of each stage between birth and adulthood, in hours.				Adult to 1st brood.	Intervals between broods, in hours.									Last brood to death in hours.	Life span in days.
	1st.	2nd.	3rd.	Total.		1st to 2nd.	2nd to 3rd	3rd to 4th.	4th to 5th.	5th to 6th.	6th to 7th.	7th to 8th.	8th to 9th.	9th to 10th.	10th to 11th.	
1	6.50	8.50	9.50	24.50	24.00	24.00	25.19	24.33	24.33	24.33	24.18	------	------	------	------	24.33	9.14
2	6.50	8.50	9.00	24.00	24.50	24.83	26.00	24.42	23.00	23.50	26.00	------	------	------	------	24.50	9.20
3	6.50	8.50	8.08	23.08	24.67	23.75	27.58	24.18	22.67	23.67	24.83	30.33	27.00	24.50	------	7.00	11.51
4	6.50	7.50	9.00	23.00	24.50	23.92	26.08	24.33	22.67	24.50	------	------	------	------	------	20.50	8.02
5	6.67	8.50	9.33	24.50	24.00	24.00	25.83	24.00	23.18	24.00	24.00	28.50	27.18	------	------	11.00	10.19
6	6.67	8.50	8.33	23.50	24.67	23.75	26.50	24.08	22.67	24.00	24.00	29.67	27.33	24.50	25.50	12.00	13.01
7	6.50	8.50	9.50	23.50	24.00	24.83	26.08	24.08	24.25	23.50	25.50	------	------	------	------	24.50	9.18
8	6.50	8.67	9.33	24.50	24.33	26.50	24.00	24.33	24.00	25.58	27.25	------	------	------	------	1.00	8.40
9	6.50	8.50	9.83	24.83	24.67	23.75	25.18	24.08	24.33	23.18	------	------	------	------	------	.50	7.11
10	6.08	8.50	10.67	24.75	24.50	23.67	25.67	24.18	22.00	23.50	24.50	25.00	27.00	24.50	25.00	25.00	13.30
11	6.08	8.67	10.50	25.25	26.00	24.00	25.50	23.50	23.50	23.18	24.85	------	------	------	------	.50	8.18
12	7.08	9.18	9.00	25.26	24.33	23.33	26.18	24.18	23.83	23.83	25.00	27.50	------	------	------	1.50	9.37
13	7.83	8.67	9.50	26.00	24.18	23.83	26.00	24.18	23.33	------	------	------	------	------	------	0.00	6.15
14	7.25	8.67	11.00	26.92	24.00	23.50	25.18	24.00	22.67	23.67	26.50	------	------	------	------	20.50	9.04
15	7.08	8.50	10.50	26.08	23.50	23.50	25.18	------	------	------	------	------	------	------	------	13.33	4.23
16	7.08	9.67	9.50	26.25	24.50	23.50	25.18	24.00	23.50	22.83	27.00	------	------	------	------	7.00	8.49
17	7.08	8.00	9.67	24.75	24.00	23.50	26.00	24.00	33.50	23.00	24.92	------	------	------	------	0.00	8.07
18	6.75	7.50	11.00	25.25	23.50	23.67	25.85	27.50	------	------	------	------	------	------	------	12.00	5.74
19	6.75	9.67	10.50	26.92	24.50	23.33	25.00	24.00	24.18	25.83	------	------	------	------	------	5.00	7.37
Minimum	------	------	------	23.00	------	------	------	------	------	------	------	------	------	------	------	------	4.23
Maximum	------	------	------	26.92	------	------	------	------	------	------	------	------	------	------	------	------	13.30
Average	6.73	8.56	9.67	24.89	24.33	23.96	25.69	24.29	23.38	23.75	25.27	28.24	27.13	24.50	25.25	11.06	8.72

The difference is due to food conditions, as will be explained later.

Incubation period of parthenogenetic eggs.—No attempt was made to study definitely the maturation division in both parthenogenetic and sexual eggs. However, Allen and Banta (1929) state, "In both the parthenogenetic and sexual egg the maturation division occurs within five minutes after egg laying. About five minutes before a clutch of parthenogenetic eggs is laid, the mother molts and discharges from the brood chamber the preceding clutch of young. The ripe eggs may thus be fixed in the mother's ovary immediately after the discharge of the young."

Thus the incubation period of the parthenogenetic eggs, that is, the time required for development from the egg laying in the brood chamber from the germarium to the extrusion of the young, can be approximated as between the last molting of the nymphal instar and the extrusion of the first brood and the intervals between broods thereafter. In Tables 1 and 2 the incubation periods may be represented as from the last molt of the postembryonic stage to the first brood, and from the first brood to the second, and so on, which show a variation of from 23.28 to 28.24 hours in soil-mungo medium and from 20.51 to 26.44 hours in soil-manure.

INCUBATION PERIOD OF SEXUAL EGGS [4]

The sexual egg differs from the parthenogenetic in the length of the incubation period. It develops a conspicuous egg case, the ephippium, which is dropped at random by the mother on the bottom of the medium. The length of the incubation period depends upon the environment, since the egg is protected by the ephippial case and can withstand for a long time desiccation and considerable pressure, especially during the dry season. In the laboratory the eggs were dried at a temperature of about 40° C. in an insect drier for ½ month. Twenty egg cases each containing two eggs were transferred individually to twenty vials containing soil-manure culture medium. Only 24 eggs hatched from 2 to 5 days thereafter; the rest failed to hatch.

It is probable that in nature *M. macrocopa* is capable of producing two kinds of sexual eggs, those which require a long resting period and those which do not. Wood (1932) found that twenty of twenty-four fertilized eggs (83 per cent) hatched without

[4] No detailed life-history study was made of the sexual mother.

TABLE 2.—*Life span, interval between birth and adulthood, and length of reproductive period in soil-manure culture medium.*

Culture No.	Duration of each stage between birth and adulthood, in hours.				Adult to 1st brood.	Intervals between broods, in hours.			
	1st.	2nd.	3rd.	Total.		1st to 2nd.	2nd to 3rd.	3rd to 4th.	4th to 5th.
20	6.50	8.00	9.50	24.00	20.00	21.50	22.00	23.33	23.83
21	6.50	8.00	9.50	24.00	21.50	20.50	20.50	26.00	25.00
22	6.50	8.00	10.00	24.50	22.00	20.50	20.50	25.50	25.50
23	6.50	8.00	9.50	24.00	20.50	21.00	20.00	24.50	24.50
24	6.67	8.00	9.50	24.17	21.50	21.50	20.19	24.30	21.50
25	6.67	8.00	10.00	24.67	22.00	20.00	20.50	24.50	24.50
26	6.50	8.00	10.00	24.50	21.00	20.50	20.00	24.50	25.00
27	6.58	8.00	10.00	24.58	20.50	20.50	20.50	26.00	25.00
28	6.50	8.00	9.50	24.00	20.50	20.00	20.00	24.50	25.00
29	6.50	8.00	9.00	23.50	20.00	20.00	20.50	24.50	25.00
30	6.50	8.00	10.00	24.50	20.00	21.00	20.50	24.50	24.50
31	6.50	8.00	10.00	24.50	21.00	21.00	21.00	24.00	27.00
32	6.50	8.00	10.00	24.50	20.00	21.00	20.50	24.00	24.50
Minimum				23.50					
Maximum				24.67					
Average	6.53	8.00	9.73	24.26	20.81	20.69	20.51	24.63	24.68

Culture No.	Intervals between broods, in hours.								Last brood to death, in hours.	Life span in days.
	5th to 6th.	6th to 7th.	7th to 8th.	8th to 9th.	9th to 10th.	10th to 11th.	11th to 12th.	12th to 13th.		
20	28.00	25.00	23.50	27.50	25.00	23.00	25.00	----------	2.00	13.07
21	24.50	27.00	----------	----------	----------	----------	----------	----------	2.00	7.96
22	25.00	27.00	23.50	25.50	25.00	24.50	26.00	----------	0.50	13.15
23	25.00	27.00	23.00	25.50	25.00	23.50	25.50	24.00	2.50	13.98
24	26.50	26.00	22.50	27.25	26.00	23.75	25.50	----------	0.00	12.94
25	26.00	26.00	23.00	26.00	24.50	24.00	25.50	26.50	2.00	14.15
26	26.00	25.50	23.00	27.33	26.00	23.00	26.00	----------	0.00	13.01
27	24.50	26.00	23.50	24.50	25.50	22.50	25.00	22.00	8.50	14.09
28	26.00	27.00	23.00	26.00	24.00	24.00	25.50	----------	4.00	13.06
29	26.50	25.00	22.50	27.00	25.50	23.50	24.00	----------	12.00	13.31
30	24.50	26.00	22.50	27.00	26.00	23.00	26.50	----------	1.50	13.00
31	27.00	26.00	22.50	26.75	25.00	23.75	26.00	----------	2.00	13.23
32	26.50	26.50	22.50	27.00	24.00	----------	----------	----------	20.50	11.73
Minimum	----------	----------	----------	----------	----------	----------	----------	----------	----------	7.96
Maximum	----------	----------	----------	----------	----------	----------	----------	----------	----------	14.15
Average	25.85	26.15	22.92	26.44	25.13	23.50	25.50	24.17	4.42	12.82

TABLE 3.—*Number of young and proportion of sexes produced by parthenogenetic females in soil-mungo medium.*

Culture No.	1st brood.		2nd brood.		3rd brood.		4th brood.		5th brood.		6th brood.	
	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.
1	15	0	0	10	16	0	20	0	19	0	13	0
2	17	0	0	15	16	0	15	0	0	17	12	0
3	14	0	14	0	12	0	1	17	14	0	0	13
4	17	0	2	15	14	0	16	0	0	15	10	0
5	18	0	0	11	16	0	14	0	0	20	9	0
6	16	0	14	1	15	3	13	0	18	0	12	0
7	17	0	0	13	15	0	0	11	0	9	14	0
8	16	0	0	16	0	15	16	0	0	18	7	0
9	16	0	0	16	16	0	4	14	0	14	13	0
10	13	0	17	0	14	2	0	12	17	0	0	12
11	14	0	15	0	16	0	0	16	18	0	7	0
12	15	0	17	0	0	16	11	0	0	22	8	1
13	14	0	17	0	1	15	19	0	0	17	0	0
14	0	14	15	0	17	0	17	0	0	15	10	0
15	0	14	17	0	18	0	0	0	0	0	0	0
16	14	0	17	0	16	0	0	18	19	0	10	0
17	13	0	14	0	0	11	17	0	0	16	9	0
18	12	0	18	0	17	0	0	18	0	0	0	0
19	0	15	16	0	17	0	16	0	0	18	12	0
Total	241	43	193	97	236	62	179	106	105	181	146	2
Minimum												
Maximum												
Average brood	14.94		15.26		15.68		18.8		16.89		10.1	
Percentage of males	15.1		33.4		20.1		37.5		63.3		15.1	

Culture No.	7th brood.		8th brood.		9th brood.		10th brood.		11th brood.		Total number of each sex.		Total number of young.
	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	
1	19	0	0	0	0	0	0	0	0	0	102	10	112
2	8	0	0	0	0	0	0	0	0	0	68	32	100
3	16	0	12	0	0	11	15	0	0	0	98	41	139
4	0	0	0	0	0	0	0	0	0	0	59	30	89
5	13	0	13	0	18	0	0	0	0	0	101	31	132
6	14	0	0	14	17	0	18	0	0	10	137	28	165
7	18	0	0	0	0	0	0	0	0	0	64	33	97
8	14	0	18	0	0	0	0	0	0	0	71	49	120
9	0	0	0	0	0	0	0	0	0	0	49	44	93
10	12	0	0	15	14	0	12	0	10	0	109	41	150
11	11	0	0	0	0	0	0	0	0	0	81	16	97
12	24	0	14	0	0	0	0	0	0	0	89	39	128
13	0	0	0	0	0	0	0	0	0	0	51	32	83
14	20	0	0	0	0	0	0	0	0	0	79	29	108
15	0	0	0	0	0	0	0	0	0	0	35	14	49
16	12	0	0	0	0	0	0	0	0	0	88	18	106
17	15	0	22	0	18	0	0	0	0	0	108	27	135
18	0	0	0	0	0	0	0	0	0	0	47	18	65
19	15	0	14	0	0	0	0	0	0	0	90	33	123
Total	211	0	93	29	67	11	45	0	10	10	1,526	565	2,091
Minimum											35	10	49
Maximum											137	49	165
Average brood	15.1		15.25		15.6		15.0		10.0		14.9		111
Percentage of males	0		23.8		14.1		0		50.0		22.24		

TABLE 4.—*Number of young and proportion of sexes produced by parthenogenetic females in soil-manure medium.*

Culture No.	1st brood.		2nd brood.		3rd brood.		4th brood.		5th brood.		6th brood.		7th brood.	
	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.
20	14	0	29	0	29	0	0	28	15	0	0	20	0	7
21	13	0	29	0	23	0	25	0	0	13	16	0	7	0
22	11	0	30	0	24	0	0	28	14	0	12	0	16	0
23	14	0	25	0	31	0	0	28	16	0	19	0	0	11
24	12	0	32	0	28	0	0	28	19	0	15	0	0	6
25	14	0	28	0	29	0	0	28	19	0	0	15	10	0
26	15	0	29	0	25	0	0	28	17	0	1	13	0	11
27	14	0	5	24	26	0	0	28	0	10	16	0	0	4
28	14	0	29	0	28	0	0	29	15	0	0	15	0	6
29	16	0	28	0	24	0	0	22	0	20	13	0	14	0
30	14	0	29	0	26	0	0	29	19	0	15	0	0	13
31	15	0	24	0	26	0	0	26	16	0	17	0	0	9
32	14	0	28	0	30	0	0	27	18	0	0	14	12	0
Total	180	0	345	24	349	0	25	329	168	43	123	77	59	66
Minimum														
Maximum														
Average brood	13.85		28.38		26.85		27.23		16.23		15.38		9.62	
Percentage of males	0		6.5		0		92.6		23.08		38.5		52.8	

Culture No.	8th brood.		9th brood.		10th brood.		11th brood.		12th brood.		13th brood.		Total number of each sex.		Total number of young.
	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.			
20	9	0	0	14	9	0	10	0	0	14	0	0	115	83	198
21	0	0	0	0	0	0	0	0	0	0	0	0	113	13	126
22	0	4	13	0	0	10	23	0	0	0	0	0	143	42	185
23	8	0	0	18	8	1	21	0	0	10	4	0	146	68	214
24	8	0	14	3	10	0	19	0	0	7	0	0	157	44	201
25	0	4	13	0	0	13	24	0	0	8	3	0	140	68	208
26	0	4	0	19	6	0	23	0	0	4	0	0	115	79	194
27	9	0	0	17	16	0	26	0	0	7	0	3	112	93	205
28	0	6	9	0	19	0	0	10	0	8	0	0	114	74	188
29	0	7	10	0	12	0	21	0	10	0	0	0	148	49	197
30	7	0	11	8	0	9	17	0	0	7	0	0	147	57	204
31	6	0	0	10	14	0	18	0	0	9	0	0	136	53	189
32	0	5	17	0	0	12	0	0	0	0	0	0	119	58	177
Total	47	30	87	89	94	45	202	10	10	74	7	3	1,706	780	2,486
Minimum													112	13	126
Maximum													157	93	214
Average brood	6.41		14.92		11.58		19.27		8.4		3.33		15.44		191
Percentage of males	38.9		50.6		32.5		4.7		88.09		33.3		31.38		

having been dried, and three that had not hatched after a week of daily examination were put out in a bottle to dry. Five weeks later, when put in water, all three hatched. The twenty-fourth egg was discarded. She tried a second group of sixty sexual eggs and a third group of sixty-seven eggs from the same line, of which 77 and 78 per cent, respectively, hatched without having been dried.

To ascertain the time required for sexual eggs to hatch without drying, twenty-five newly extruded ephippial egg cases were transferred each to a vial containing horse-manure medium. Two to twenty-two days later twenty hatched. The remaining eggs were dried for five days, after which they were transferred to a fresh medium, where six young developed; the rest were regarded as having been disintegrated. Thus this species seem to maintain itself, in the Philippines as well as in the United States, regardless of whether or not the body of water in which it lives dries up.

MALE

Like the young parthenogenetic female, the newly-born male readily swims about in the medium, seemingly searching for food. Its first instar is from 0.4 to 0.45 millimeter long, and the second from 0.44 to 0.55 millimeter. It is hyaline in color. It molts twice before it becomes an adult. The first instar occurs after about 7 to 8 hours, and the second takes about 8 to 9. Molting takes place every 18 to 21 hours thereafter. The life span of the male is from 5 to 9 days in the soil-manure medium, about 6.9 days on the average.

MOLTING

Molting is essentially the same in all sexes and stages. When the adult female molts, the middorsal line of the body splits for the exit of the young, the ephippium, or the body of the animal itself. Shortly after the extrusion of the young or the ephippial eggs, the animal whirls around vigorously untill the exuvia is completely separated from the body. The animal has no fixed place for molting, as it constantly moves about, presumably looking for food.

COPULATION

The male, after the second molting, begins to copulate with the sexual female who becomes mature after the third molting. As observed in the culture experiment, when the male was intensely excited he made a few abortive attempts before he succeeded. With his antennules, he seized any female, whether

sexual or parthenogenetic, and at first clasped her at any part of her body. When the female was a parthenogenetic one, she fought vigorously to get free, and the male soon released her. If the female was a sexual one ready for mating, he stayed sufficiently long to complete copulation. In copulation, the male slightly opens his carapace and takes hold of the female. The female becomes motionless, allowing the male to adjust himself, until he reaches the anterior margin of one side of the female. He then bends his abdomen ventrally, so that it extends posteriorly to between the ventral margins of the female's carapace. In this position he moves his abdomen about, presumably ejecting his spermatozoa. After the male has adjusted himself the female carries him about. The mating lasts about 15 minutes.

PROPORTION OF SEXES

The proportion of sexes in moina is very difficult to determine, as it is controlled by environmental factors; namely, food, crowding, temperature, and others. Working on *Moina rectirostris*, Grosvenor and Smith (1933) concluded that by isolating the parthenogenetic females at birth until the extrusion of the brood at a temperature of 25° C. to 30° C., the production of sexual forms is entirely suppressed. In their experiment parallel cultures of related females crowded together in the culture glasses at a temperature of 25° C. to 30° C. gave 30.3 per cent males, and at about 14° C. gave 52.3 per cent males; isolated females at 14° C. gave 19.1 per cent males, and in an ice chest at about 5° C., 5.4 per cent males, and crowded together, they gave 42.5 per cent.

According to Stuart and Cooper (1932), isolated moina mothers reared in 500 ml of highly diluted medium will occasionally produce males; a low percentage of males in small broods of young and retardation of mothers in diluted medium make it seem probable that the production of males is due to lack of food; crowded mothers reared in various dilutions of medium and with frequent changes to prevent the accumulation of excretory substances, produce males in proportion with the dilution of the medium; crowded mothers reared in constantly flowing medium produce males, provided the medium used in the experiment is sufficiently diluted.

Stuart, Cooper and Coody (1933) found that under certain experimental conditions carbon dioxide (CO_2) may be employed to suppress completely the production of male cladoceran young by crowding the mothers, while under other conditions, presum-

ably associated with depression periods, carbon dioxide is wholly ineffectual in the suppression of the male production.

Isolated mothers.—In conjunction with the experiment on the number of broods that the mothers can give during their life time, the numbers of males and females were also determined to study the proportion of sexes by the isolated mothers during their life time. To determine the optimal and minimal food conditions for the organisms, two media were used, shown in Tables 3 and 4. Table 3 shows that the average percentage of males in the first brood was 15.1, in the second 33.4, in the third 20.1, in the fourth 37.5, in the fifth 63.3, in the sixth 15.1, in the seventh 0, in the eighth 23.8, in the ninth 14.1, in the tenth 0, and in the eleventh 50, with the total average percentage of 22.24. The lowest percentage of males, in the seventh and tenth broods, was 0, and the highest, in the fifth brood, was 63.3.

A study of the total numbers of each sex produced by every mother during her life time shows that the ratio of males to females is very variable, with the females always outnumbering the males.

In soil-manure medium (Table 4) the proportion of males to females produced during the life spans of thirteen females was slightly greater than in cultures reared on soil-mungo medium. The average percentage of males in the brood of the first was 0; in that of the second, 6.5; in that of the third, 0; in that of the fourth, 92.6; in that of the fifth, 23.08; in that of the sixth, 38.5; in that of the seventh, 52.8; in that of the eighth, 38.9; in that of the ninth, 50.6; in that of the tenth, 32.5; in that of the eleventh, 4.7; in that of the twelfth, 88.09; and in that of the thirteenth, 33.3. The total average percentage was 31.38. Here again we found that during the life spans of all the mothers the females always far outnumbered the males, the minimum numbers of males produced being 13, and the maximum, 93.

Crowded mothers.—The experiment on crowded mothers (Table 5) was carried only to the fourth brood, since the number of mother in every bottle was gradually decreasing. In the brood of the first mother, the average percentage of males was 22.64; in that of the second, 37.95; in that of the third, 41.44; and in that of the fourth, 49.33. The total percentage was 36.94. The total number of young produced by parthenogenetic females crowded together in bottle at room temperature was 2,473 females and 1,446 males. The males amounted to 36.94 per cent of the total number of young produced. The percentage of males produced by the isolated mothers is 31.38, against 36.94 per cent

TABLE 5.—*Proportion of sexes produced by crowded mothers.*

Culture No.	First brood.				Second brood.			
	Female.	Male.	Average brood.	Average of male.	Female.	Male.	Average brood.	Average of male.
				Per cent.				*Per cent.*
101	90	17	10.70	15.90	85	41	12.60	32.50
102	100	20	12.00	16.60	71	34	11.70	32.40
103	105	17	12.70	13.90	57	55	12.40	49.10
104	90	15	10.50	14.30	53	45	12.25	45.90
105	69	25	9.40	26.90	111	53	16.40	32.30
106	76	40	11.60	34.40	83	117	20.00	58.5
107	104	25	12.90	19.30	79	73	15.20	48.00
108	65	43	10.80	39.80	155	8	16.30	4.90
Total and average	699	202	11.33	22.64	694	426	14.61	37.95

Culture No.	Third brood.				Fourth brood.				All broods.			
	Female.	Male.	Average brood.	Average of male.	Female.	Male.	Average brood.	Average of male.	Female.	Male.	Average brood.	Average of male.
				Per cent.				*Per cent.*				*Per cent.*
101	39	56	11.80	58.80	58	22	13.30	27.50	272	136	12.14	33.33
102	47	30	12.80	38.90	37	43	13.30	53.75	250	127	12.45	33.25
103	49	43	13.10	46.80	44	25	11.50	36.20	255	140	12.85	37.45
104	42	55	13.90	56.70	25	46	11.80	64.50	210	161	12.11	43.39
105	124	50	19.90	28.70	57	90	21.00	61.20	361	218	16.68	37.65
106	163	17	22.50	9.40	86	66	19.00	43.43	408	240	18.28	37.04
107	114	59	19.22	34.10	65	72	18.40	52.50	362	229	16.43	38.75
108	58	83	20.10	58.10	72	61	19.00	45.60	350	195	16.55	35.77
Total and average	629	393	16.68	41.44	442	425	15.91	49.33	2,473	1,446	14.69	36.94

produced by crowded mothers. It is most probable that the number of males increases proportionally with the density of the population under fixed environmental conditions.

NATURAL HABITAT

Moina macrocopa lives in filthy and dirty environment throughout the year, especially in fresh-water pools polluted with decomposing material. It may be found in stagnant pools and ditches near or under houses, where food particles, dead grass, decaying wood, empty cans, manures, and other refuse which constitute the food necessary for the development of microörganisms, are thrown.

Intermittent rain during the rainy season has some influence on the abundance of moina in nature. The rain makes the water in the pools rise up to a higher level. It brings in food material from various sources, thus causing rapid multiplication of moina and other organisms living in the pools. After a few days, when much water in the pools has already evaporated, part of the moina population dries up, the remainder becoming overcrowded while the food supply becomes exhausted, causing death to many waterfleas and other organisms, which, however, leave sexual eggs in the pools for development under more favorable conditions. However, as long as there is water and food constantly supplying the pools, these organisms may continue to live there throughout the year.

ENEMIES

Like other animals the moina is beset by many enemies. It was observed that related crustaceans, like the cyclops, crowd out the moina; that the hydra first paralizes and later destroys this species, in spite of its size and strength; that *Vorticella* sp., and Rotifera, notably *Brachionus rubens,* sometimes use the body of the moina as a resting place, covering it almost entirely and thus depriving it of food and hindering its movement; and that bacterial masses and molds entangle it to such a degree as to cause its death. It constitutes the food of many kinds of fishes.

Either by actual separation or avoiding adverse conditions, such as acid media and pollution, these dangers may readily be removed from moina collected for aquarium purposes. Hydra may be killed by immersing the entire catch in 1 per cent salt solution (Viehoever, 1935). Since *B. rubens* goes with the catch from nature, it may be separated by transferring the

catch to a large amount of tap water and selecting from it those individuals of moina suitable for culture. Filtering the water before use may help eliminate the infestation of cyclops, Rotifera, and *Vorticella.*

FEEDING

The method of feeding was actually observed with naked eyes and with the use of a 14x hand lens when the animal was freely swimming in the culture medium, and a low-power Zeiss dissecting microscope when it was placed in an uncovered cell deep enough to allow it free play. In describing this process the writer was guided by Hartog's (1902) very illuminating description of the feeding of phylopods and Cladocera.

In feeding, the animal may be seen chasing from the top to the bottom or vice versa, with brisk jerky motions, stopping on the bottom surface, at the inside, of the glass, at the top margin, or at the surface film of the medium, with the cilia of the limbs in constant vibration. The thoracic appendages cause the flow of the water into the shell through the angles on either side between the carapace (Plate 1, fig. 7, *ca*) and the head. Here the flow of water passes the antennules (Plate 1, fig. 7, *ant*) which are thus in a very good position as sense organs for perceiving any change in the properties of the indrought. The stream flows backward between the valves in the spaces traversed by the limb proper. Here the limbs (Plate 1, fig. 7, *ft*), which are armed with rows of ciliated setæ, are efficient in producing current. The food in suspension drifts into the angle where the hind end of the abdomen bends abruptly down, as it passes back. Here the food is turned onward and then under, and along the medium line of the body till it reaches the oral region behind the labium where the play of the mandibles sends it to the gullet to be swallowed.

HYDROGEN-ION CONCENTRATIONS

According to Herms (1929) many species of organisms seem to require a certain hydrogen-ion concentration range in the medium in which they live. During the course of his investigations on mosquitoes he found that *Theobaldia incidens* tolerated a pH range of from 5.4 to 7.6, and *Anopheles maculipennis* a pH range over 7, that is, from 7.2 to 8. Chipman (1934) states that the pH of the cotton-seed meal medium for culturing *Daphnia* was adjusted to 7.2, with the addition of sodium carbonate,

and he was able to grow the organisms rapidly and to produce normal clones.

Since pH is said to be important to the lives of organisms, experiments on pH concentration were conducted with soil-manure culture medium.

To determine the pH concentration range in relation to the growth of *M. macrocopa,* examination was made of the pH concentrations of various pools where moina were found growing profusely, and of that of the media used for culturing the organisms. The pH concentration of samples taken from a pool in Pasay, Rizal Province, was 8; back of Philippine Women's University, Manila, 7.5; and in the basement of the Bureau of Science, 9. Soil-mungo culture medium showed a pH concentration of 7; soil-soybean, 7.1; soil-ricebran, 6.8; yeast, 7.8; and soil-manure, 7.5.

In the study of the pH concentration range two main factors were determined; first, the maximum pH concentration in acid (Table 6), and second, in alkaline (Table 7). To determine the pH range in acid, eight similar-sized bottles, 2½ inches in diameter and 5 inches high, with an opening of 1⅜ inches in diameter, numbered 1 to 8 and each containing 100 cc of soil-manure medium, were used. Relatively proportional amounts of sulphuric acid ($\frac{n}{10}$ H_2SO_4) solution were added to the medium in all bottles except bottle 1, which was used as check. Six mother moina were introduced into each bottle. The results are noted in Table 6.

TABLE 6.—*pH in relation to growth of Moina macrocopa in acid soil-manure medium.*

Bottle No.[a]	$\frac{n}{10}$ H_2SO_4 solution added.	pH range of medium.		Population.	Condition of moina.
		Newly treated.	4th day.		
	cc.				
1	0.00	7.60	7.60	160	Vigorous, active, reddish.
2	2.28	7.28	7.20	154	Do.
3	2.54	6.85	6.70	165	Do.
4	2.80	6.52	6.40	155	Do.
5	3.07	5.86	6.10	165	Do.
6	3.20	5.50	5.80	160	Do.
7	3.35	5.20	5.50	145	Moved slowly, often stayed on the bottom for about 3 hours, then gradually became active again.
8	3.60	5.00	----------	----------	Moved slowly and died in about 3 hours.

[a] Each bottle contained 6 moina in 100 cc of medium.

The experiment in alkaline media was similarly conducted. Bottle 10 remaining as check, to bottles 11 to 18 was added sodium hydroxide ($\frac{n}{10}$ NaOH) solution in gradually increasing concentrations. The results are given in Table 7.

The results of the experiments clearly indicate that the organisms can tolerate a pH range of from 5.2 to 9.2 in a solution and that they produce normally in spite of the variations in the pH concentrations of the medium.

TABLE 7.—*Growth in relation to pH range of Moina macrocopa in alkaline on soil-manure culture medium.*

Bottle No.[a]	$\frac{n}{10}$ N_aOH solution added.	pH range of medium.[b]		Population.	Condition of moina.
		Newly treated.	4th day.		
	cc.				
10	0.00	7.60	7.60	165	Vigorous, active, reddish.
11	3.46	7.80	7.70	160	Do.
12	3.60	8.10	7.90	150	Do.
13	3.80	8.30	8.00	155	Do.
14	3.98	8.60	8.20	160	Do.
15	4.15	8.80	8.30	170	Do.
16	4.30	9.00	8.50	140	Do.
17	4.50	9.20	8.80	150	Moved slowly and gradually became active again.
18	4.67	9.40	----------	----------	Died in 12 hours.

[a] Each bottle contained 6 moina in 100 cc of serum.

[b] All pH concentrations were read by Dr. M. M. Alicante, soil biologist of the Bureau of Science, with the use of a colorimeter.

FACTORS IN LABORATORY CULTURE

Water.—The selection of a suitable water supply is of prime importance for the success of a culture medium. Rain water was found to be excellent. The water from fishponds near the Bureau of Science, Manila, in which algæ and other water plants grow, has been tried with success after filtration through filter paper. Two days after it has been chlorinated, tap water was found excellent for the breeding of moina. It was not observed, however, that newly chlorinated tap water killed the waterfleas.

In moina culture it seems that brownish yellow color indicates optimal conditions for the progress of the desired pollution. As a rule the writer has obtained best results when the media were yellowish, like those of horse manure, and yeast added with ferric chloride or ferrous sulphate. Some culture media containing soy-bean meal, mungo meal, rice bran, and yeast,

are practically colorless. However, in nature the water where the moina grows is sometimes clear and colorless.

Oxygen.—It is generally observed that a moina culture, especially in dense growth, consumes large amounts of oxygen. Many collectors have experienced that crowded moina in their containers do not live five hours. In the United States, in collecting daphnia during warm weather, the collectors, knowing that warm water holds less oxygen than cold water, place cakes of ice in the pails containing daphnia to insure the live delivery of the catch. Although in nature the large and highly efficient breathing system furnishes oxygen to moina even in highly polluted water or at the bottom of the pools, they can live on little oxygen. Moina can live in a tightly corked vial containing 5 cc of water for days.

Kerosene spread out on the surface of the water retards the absorption of oxygen from the air. Kerosene spread over natural pools for killing mosquito larvæ did not seem to affect the growth of moina, except when the oil came in contact with the animals. In this connection an experiment was performed in order to test the effect upon moina of kerosene spread on the pool. October 4, 1934, two beakers were filled with the usual concentration of soil-mungo medium. Ten mother moina were introduced into each beaker and the medium was entirely covered with kerosene. In the culture, which was kept up until October 15, 1934, the organisms lived and grew as well as in a big aquarium, cultured simultaneously.

Temperature.—No study has been made as to the temperature best suited for the growth of moina. However, the temperature of the water in which the organisms grow well, both in nature and under laboratory conditions, was found to be from 26° to 31°C.

Light.—In moina culture diffused light is found to be best. According to Viehoever (1935) direct sunlight keeps the growth of bacteria and protozoa in check, thus preventing the excessive growth in certain culture media, although it does stimulate the growth of algæ.

Containers.—Moina can be cultured in various containers, from narrow-mouthed to wide-mouthed jars. In the course of observation many sizes of containers were used. In most cases shell vials of $1\frac{1}{2}$ by 8 to $2\frac{1}{2}$ by 8 centimeters, various sizes of specimen jars, museum jars, aquaria, enamel basins, old cement

tanks, and beakers, were found satisfactory. For mass production the writer used aquarium jars containing from 4 to 65 liters of water, old cement tanks of 15 liters, and fruit jars of 1 liter, with satisfaction. The culture containers, tools, and other culture materials should be carefully cleaned and kept away from poisonous materials.

Selection of healthy breeders.—Groups of moina newly taken from nature exhibit various sizes and colors. They are large and small, yellowish to reddish. The bigger and seemingly healthy individuals with reddish color are best for breeders.

Food.—Food is the most important factor in the laboratory breeding of moina. Food must be always available. Infrequent feeding may lead to the development of males and ephippial females, although over-abundance is equally detrimental.

A mixture of changeable and uncontrollable organisms in raw cultures serves as a source of pure culture. Such cultures of chemically and biologically changing composition are obtained upon suspensions of manure, soil, mungo, rice bran, soy bean, and other media, in water, or setting aside rice-straw infusion. They provide culture media for a host of microörganisms, like bacteria and protozoa, that live on the dissolved food nutrients, such as fats, proteins, carbohydrates, and mineral salts.

Banta (1921) ascertained that daphnia may be fed upon a mixture of various protozoa and protophyta from the sediment of ponds containing an abundance of organic matter, upon bacteria, and upon certain unicellular green algæ.

For food, bacteria have been emphasized by various workers who have observed the condition under which moina and daphnia breed in nature as well as in the laboratory. Treillard (1925) claimed that he propagated daphnia in pure cultures of bacteria. Stuart, McPherson, and Cooper (1931) experimented with various kinds of bacteria as food organisms.

Knowing that in many of the manural media urea bacteria would be present, Viehoever (1935) prepared a mineral medium containing urea consisting of 0.02 per cent Liebit extract added to an aqueous solution of K_2HP_4, 0.1 per cent, $MgSO_4$, 0.03 per cent, NaCl, 0.01 per cent, and $FeCl_3$, 0.001 per cent, in which urea bacteria readily grow. While the sterile solution in various concentrations was unsatisfactory, two drops of this selective urea with urea bacteria added to a 30-cc culture of tap water, exposed to direct and diffused sunlight, gave fair to good growth of daphnia.

In view of the fact that certain kinds of bacteria are necessary as food for moina, bacterial analyses [5] of the two most promising of the culture media were made. The bacteria found in soil-manure culture medium were *Flavobacterium aurescens* (Ravenel), *Flavobacterium diffusun* (Frankland), *Bacterium lactis ærogenes* Escherich, and *Micrococcus flavus* (Lehmann), and in soil-mungo culture medium, *Flavobacterium aurescens* (Ravenel), *Micrococcus auranticus* (Schröter) Cohn, and *Bacterium lactis ærogenes.* The number of species thus far found is not definite, since the materials and the media were freely exposed to contamination. No attempt was made to determine which of the bacteria was the best food for *Moina macrocopa.*

The writer used algæ from fishponds for moina culture, after straining the pond water through muslin, but unfortunately the waterfleas did not grow as well on this medium as on soil manure, and lost their natural color. In old yeast, soil-soybean, soil-mungo, and soil-ricebran media, algæ grow at the side and on the bottom inside the aquaria. Moina grows and multiplies in those media, but not as well as in nature. In my experiments, moina grew in yeast, but the offspring became whitish.

COMPARATIVE STUDY OF CULTURE MEDIA

Banta (1921) announced a convenient culture medium for daphnids in the form of soil-manure. Chipman (1934) believes that a satisfactory culture medium having several advantages over the manure infusion is prepared of 1 liter of filtered pond water, 90 grams of garden soil, and 17 grams of cotton-seed meal for stock solution. Bond (1934) announced that ordinary Fleischmann's yeast had been fed for some months to mass cultures of *Daphnia magna* and *Artemia* with excellent results, reproduction and growth being markedly more rapid and population more dense than in any of the usual media, but that it is necessary to have a stream of air bubbling through the medium at all times or the yeast may prove lethal, probably because it gives off carbon dioxide. Weibe (1930) found soy-bean meal a good fertilizer for the production of organic matter in ponds.

Since pond water, as suggested by Banta (1921) and Chipman (1934) is not available throughout the year in Manila, tap water

[5] Bacterial analyses were made by Dr. M. S. Basaca, bacteriologist, Bureau of Science.

was used. According to the analysis of the Metropolitan Water District, tap water is soft, because it contains little bicarbonate, calcium, and magnesium, about 90 parts per million, as calculated from magnesium and calcium content.

In some of the experiments reported below, garden soil was used. According to the analysis made by Mr. Francisco Tantoco, Bureau of Science, the total amount of organic matter in garden soil is 6.09 per cent,—nitrogen 0.078, phosphorus 0.16, calcium 4.75, magnesium 1.27, and potassium 0.327.

Carbohydrates (*gaogao*) (*experiment 1*).—September 20, 1935, ½ gram of gaogao was mixed in 500 cc of distilled water and boiled for 30 minutes. The mixture was divided into two equal parts and distilled water added to make 900 cc in each of two battery jars. The jars were covered with cotton and were autoclaved for 25 minutes under a pressure of 20 pounds. The solution was set aside to cool till morning. Five mother moina were washed in sterile water and introduced into each jar, which was always kept covered. After about 24 hours all mother moina had died, leaving a few young which died the following day without showing perceptible growth.

Soil-soybean culture medium (*experiment 2*).—September 4, 1934, a stock medium was prepared in proportions of 10 grams of soy-bean meal to 1 liter of water, boiled for 25 minutes, and left to cool in a covered beaker for about 24 hours, after which 250 grams of garden soil were added. After thorough stirring, the mixture was loosely covered and set aside in a dark place at room temperature for three days. During this period the mixture fermented and produced considerable gas. At the end of three days the supernatant liquid was decanted and strained off through muslin. The stock solution was restrained, to eliminate any bacterial masses that might have developed.

To determine the right concentration of this medium for rearing moina, stock solution in amounts of 35, 40, 45, and 50 cc was added consecutively each to one of four aquarium jars containing four liters of water each. Ten moina were introduced into each jar. Observation showed that in strong concentrations of the medium bacterial masses formed, either in suspension, at the surface of the medium, or at the side of the jar, interfering with the free movement of the moina and often causing their death, but in a dilution of 35 to 40 cc of strained

stock solution added to four liters of water a final dilution was obtained which remained quite clear and in which moina grew. The medium was replenished with 15 cc of strained stock solution after the fourth day. It has been desirable to renew the medium in which the animals were growing at intervals of a week or more. Fresh solution was prepared every week.

In the mass-production experiment fresh catches of moina living under natural conditions were used. Fifty healthy and vigorous mother moina were introduced into each of two aquaria containing 15 liters of water each of the culture medium. As the animals grew in the medium, they gradually became whitish, and their growth was not so dense. The culture was tried with similar results for a month in three old cement tanks each containing 15 liters of culture medium.

Soil-ricebran culture medium (*experiment 3*).—September 17, 1934, a stock solution was prepared in proportions of 10 grams of rice bran to 1 liter of water, boiled for 25 minutes, and left to cool in a covered beaker for about 24 hours, after which 250 grams of garden soil were added. After thorough stirring, the mixture was loosely covered and set aside in a dark place at room temperature for three days. Like the soil-soybean medium, the mixture fermented and produced considerable gas, forming supernatant liquid which was decanted, and the liquid strained off through muslin.

For determination of the right concentration of the medium the same method as that in experiment 2 above was used, and the right dilution was found to be at from 40 to 45 cc of the stock solution to 4 liters of water. The results were identical to those of the preceding experiments.

Soil-mungo culture medium (*experiment 4*).—The culture medium was prepared like the soil-soybean medium and the soil-ricebran medium. The method for determining the right concentrations was the same as that followed in the preceding experiments, and from 35 to 40 cc of the stock solution to 4 liters of water was found to be the optimal concentration. The medium seemed somewhat better than the soil-soybean and soil-ricebran media, as the growth obtained was apparently denser and the reddish coloration remained a little longer, due probably to the greater amount of iron present in mungo (Table 11).

TABLE 8.—*Number of young produced on soil-manure and yeast-culture media compared.*

Culture No.	Moina.	Date.	Medium.	Young.	
				Per mother.	Total.
		1934			
1	1	Oct. 2–9	Soil-manure	1,691	
2	1	Oct. 2–10	----do	1,766	3,457
3	1	Oct. 2–9	Yeast	484	
4	1	Oct. 2–10	----do	387	871

Yeast culture medium alone (*experiment 5*).—September 4, 1934, 150 mother moina were introduced into a uniform suspension of $\frac{1}{8}$ of a fresh cake of Fleischmann's yeast in 50 cc of water, which was poured into an aquarium containing 30 liters of water. On the fifth day the culture grew gradually denser, the animals forming schools around the sides and corners of the aquarium; thereafter the moina decreased in number. The food supply was replenished every fourth day until September 17, 1934, but no apparent increase in the density of the moina occurred. Sexual females and males seemed predominant. The coloration of the animals changed noticeably. The animals were reddish in nature, but generations bred on the medium gradually lost their color and became whitish. The experiment was repeated in a big aquarium tank containing about 65 liters and a series of six old cement tanks, each containing 15 liters of the culture medium, with identical results.

Yeast culture medium with aquatic plants (*experiment 6*).—According to Innes (1935) the main purpose of plant in aquaria is to supply oxygen, which they do under the influence of light, while at the same time they absorb the injurious gas (carbon dioxide) given off in breathing by all animals. September 18, 1934, aquatic plants (*Hydrilla* sp. and *Vallisneria* sp.) were planted in the sand on the bottom of the aquarium. The same quantity of culture medium and the same number of moina as in preceding experiments were used, with similar results.

Yeast medium with air bubbling (*experiment 7*).—The test was performed February 16, 1935, with the same number of mother moina and the same amount of culture medium used in

the preceding experiment, but with a stream of air bubbling through the medium at day time. The culture grew dense until about the seventh day, when the organisms decreased in number and became almost completely whitish. Another trial was made with similar results.

Rice-straw infusion (*experiment 8*).—The infusion was prepared in the following manner: On 10 grams of rice straw on the bottom of an aquarium jar containing 4 liters of water, several pieces of gravel were placed to keep the straw from floating. Twenty-five mother moina were introduced. The culture grew rapidly, but lasted only about five days. As the straw stayed longer in the water, molds began to grow on it and entangled the animals that happened to alight on them. The entire medium had to be changed and the receptacle well washed. Several trials were made, and it was found that more straw was unnecessary because molds and bacteria were so abundantly produced that the medium became slimy and foul in a shorter time, and thus unfavorable to the growth of the animals.

Soil-manure culture medium (*experiment 9*).—It was previously stated that soil-manure culture medium was used for culturing the animals from one clone. Banta's (1921) directions for the preparation of the medium are as follows:

> Two pounds of garden soil are placed in a large battery jar (9 in. diameter); to this are added six ounces of finely divided fresh (8 to 15 days old) horse manure and the whole is covered with 10 quarts of strained pond water. The mixture is allowed to stand at 15° to 20°C. without disturbance for three days when it is strained through silk bolting-cloth. The proper straining is facilitated by carefully dipping out and straining most of the supernatant liquid and then agitating the remainder, and with it rubbing a very small portion of the soil through the straining cloth. The solution is then ready for use, though in addition to being thoroughly stirred before being placed in the culture bottles it is usually diluted by adding pond water in the proportion of 1 to 4 to 1 to 2 depending upon the degree of density in the appearance of the solution.

For mass-production culture Banta's method of preparing the culture medium undoubtedly seems tedious and complicated to fish culturists. A much simpler method is the following: One hundred grams of garden soil and 10 grams of dried horse manure (dried for 5 days and stored for future use) are placed in a glass jar; to these are added 4 to 5 liters of water. About half a liter of the solid materials are poured in and finely broken up with the fingers or stirred vigorously with a stick; finally the rest of the water is added, and the solution is allowed to stand for a day or two. Before the solution is used, all debris floating

on top of the water should be taken off by means of a net. The sediment is allowed to remain on the bottom of the jar.

Mass-production test (*experiment 10*).—Three aquaria, each containing 50 liters of water, were prepared according to the above proportions. To each were added 1,000 grams of garden soil, and 100 grams of dry horse manure uniformly mixed with 1 liter of water. After the solution had been allowed to stand for 24 hours all floating debris was carefully removed without disturbing the sediment. To each aquarium 300 old-stock mother moina were introduced. In about 5 days the population became so dense that the animals could be seen thickly crowding in all directions of the medium. Parts of the culture were gathered every morning by means of a course conical muslin net to feed a group of 60 fishes, consisting of zebra, moon, and fighting fishes.

In order to maintain a continuous food supply for these fishes, as the thick population of the organisms remained to about the seventh day, rotation methods were used. The medium of one of the aquaria was entirely changed every six or seven days, depending upon the density of the animals, while the organisms of the others were being used for feeding.

In gathering the organisms the net should be wide and deep enough, the rim 6 inches wide and the net 2 to 5 inches deep, depending upon the size of the receptacle. The net should be dipped and moved slowly forward or sideways, and care taken not to disturb the whole medium. The organisms must be transferred immediately from the net to a receptacle containing sufficient water.

In the culture medium the organisms can be maintained for more than a month, if a crowded population is not desired, provided fresh water is added to the medium to replace that which has already evaporated, and the culture stirred slowly once a week. This method has been tested by the writer many times, both in small receptacles containing 4 liters of water and in large ones containing 50 liters.

When the medium gets old, there is a tendency for the formation of bacterial masses, molds, or hydra, at the side of the glass, which may entangle and kill the animals. It is, therefore, necessary to wipe off carefully with a piece of cloth the sides of the aquarium once every two or three days, depending upon the condition of the aquarium.

Comparative study of soil-manure and yeast culture media in number of production (*experiment 11*).—To determine whether

soil-manure or yeast medium shows the better production, one two-hour-old female moina was introduced into each of four aquarium jars, two of which contained 4 liters of horse-manure culture medium each, and two, 4 liters of yeast medium. The results are noted in Table 8. The production in yeast culture medium was comparatively small, being 871 as against 3,457 in soil-manure medium. The general appearance of the animals was whitish, and they seemed not as vigorous as those of the soil-manure culture. The result of the experiments seem to indicate that yeast alone as a food is far inferior to soil-manure in moina culture, due to deficiency in iron content, as explained below.

Restoration of color (*experiment 12*).—Since moina become whitish when cultured in soil-ricebran, soil-mungo, soil-soybean, and yeast media, tests to restore their natural color were conducted in the following manner. December 1, 1934, twenty mothers from each of these media were first washed in tap water and introduced separately into 4 glass aquaria containing 4 liters each of soil-manure medium. About 4 days after, the animals turned reddish, as in nature. The experiment was repeated three times with similar results.

THE ROLE OF IRON IN MOINA CULTURE

Both favorable and unfavorable culture media have been observed in the previous experiments, each involving a more or less complex problem. Some culture media, like yeast, grew moina, but the population became whitish and less productive; but when moina was cultured in horse-manure medium, the progeny became reddish, vigorous, and productive. What are the factors that determine these conditions? Are these conditions due to food nutrients?

In moina culture it is the purpose of the medium to provide enriching substances in solution, so as to promote the growth of microörganisms, as bacteria and protozoa. The liquid must contain organic nutrients, such as carbohydrates, proteins, and fats, that may be assimilated by unicellular organisms. Proteins are present abundantly in all but rice straw (Table 9). Carbohydrates and fats are abundant in all ingredients of soy bean and rice bran. Organic nutrients are not sufficient for bodily use; mineral salts, which are abundant in all materials, especially in rice bran, rice straw, and in horse manure, are also essential.

TABLE 9.—*Composition of culture media.*

Sample.	Moisture.	Proteins.	Fats.	Crude fiber.	Carbohydrates.	Ash.
Horse manure [a]	9.46	11.92	2.03	23.40	24.72	28.47
Yeast (Fleischmann's) [a]	69.42	16.95	1.44	1.09	9.08	2.02
Mungo [b]	9.98	24.28	1.15	3.59	57.44	3.56
Soy beans [b]	4.95	39.08	20.07	5.69	24.96	5.25
Rice bran [c]	10.04	11.27	19.08	8.67	40.62	10.32
Rice straw [d]	9.81	3.26	1.06	33.33	36.24	16.30

[a] Analyses made by Mr. E. F. Gutierrez, of the Bureau of Science.
[b] Hermano, A. J. Food values. Bur. Sci. Pop. Bull. 16 Manila (1934).
[c] West, P. A., and A. O. Cruz. Philip. Journ. Sci. 52 (1933) 1–78.
[d] Analysis made by Mr. A. O. Cruz, of the Bureau of Science.

It was found that *M. macrocopa* grew best in soil-manure medium, even though the stock had remained in the laboratory for a long period. The animals reproduced abundantly in the medium for a certain period and remained from yellowish to reddish, like in nature, while in soil-mungo, soy-bean, rice-bran, and yeast culture media the organisms gradually became whitish, indicating an unhealthy condition. To determine the necessary food elements in soil-manure and rice-straw media that result in the yellowish to reddish color of the animal, and the reason for the whitish population obtained in the other media, quantitive analyses were made of the food values of various materials. Table 9 shows that mungo, yeast, and soy bean are rich in proteins; soy bean and rice bran in fat; yeast, mungo, rice bran, and rice straw in carbohydrates. These results prove that organic nutrients had nothing to do with the reddish color and the vigor of the organisms in soil-manure. Horse manure and rice straw contain much more ash than the materials used for the rest of the media, seeming to indicate that the main cause of the reddish color was the presence of mineral salts. Upon tracing its mineral content, yeast (Table 10) was found to contain only a trace of iron.

To determine whether lack of iron was the main cause of the anæmical condition of moina, the following experiments were performed. A uniform suspension was prepared of a little yeast in 6 liters of water. Some of the mixture was placed in each of 11 bottles each containing 450 cc of water. Five bottles were numbered from 1 to 5, another five from B–1 to B–5, respectively, and the one left was used as check. With a narrow-pointed pipette 1, 2, 3, 4, and 5 drops (23 drops equal 1 cc) of ten per cent ferric chloride were introduced into the bottles of the first

group, and 1, 2, 3, 4, and 5 of ten per cent ferrous sulphate were introduced into the bottles of the second group, and the mixtures stirred well. Five mother moina were introduced into each bottle. After the third day it was observed that all adult moina in ten bottles containing the ferric compound were from yellowish to reddish, showing an increase in coloration directly proportional to the concentration of iron in the medium, while in the check the adults and young moina became whitish.

The above results indicate that iron salts, existing in organic combination in the materials fed, are necessary for the growth of moina. Analyses for iron content were made on the different materials used in the culture media. The results are shown in Tables 10 and 11.

TABLE 10.—*Analysis of the ash in yeast.*[a]

Constituent.	Per cent.
Phosphorus pentatoxide (P_2O_5)	54.5
Potassium oxide (K_2O)	36.5
Magnesium oxide (M_gO)	5.2
Calcium oxide (CaO)	1.4
Silica (SiO_2)	1.2
Sodium oxide (Na_2O)	.7
Sulphur trioxide (SO_3)	.5
Chlorine (Cl)	trace
Iron (Fe)	trace
	100.00

[a] Reported by the Fleischmann's Yeast Company according to Viehoever (1933).

TABLE 11.—*Iron contents of the materials used in moina culture.*

Samples.	Iron (F_2O_3). Per cent.
Horse manure	0.15
Yeast	0.32
Mungo	0.47
Soy bean	0.38
Rice bran	0.026
Rice straw	0.07

Table 11 shows that the iron content of horse manure is lower than that of yeast, mungo, and soy bean, but higher than that of rice bran and rice straw. Experiments 2, 3, 4, 5, 8, and 9 showed that 35 to 40 cc of soil-soybean stock medium in 4 liters of water, 40 to 54 cc of soil-ricebran in 4 liters of water, and 35 to 40 cc of soil-mungo in 4 liters of water, were the optimal concentrations; whereas in yeast medium $\frac{1}{8}$ of a cake in 30 to 35 liters of water in soil-manure, 10 grams of horse manure and

100 grams of garden soil in 4 to 5 liters of water, and rice straw 8 to 10 grams of straw in 4 liters of water, were found to be the optimal concentrations. Apparently soil manure is more concentrated than any of the media. Yeast contains proportionally more iron than horse manure when the analysis is based on ash, but in the sample the iron content is only 0.004 per cent, while in horse manure it is 0.03. Iron salts that may be taken in either directly from water or contained in the yeast plants themselves and assimilated by the organism do not seem sufficient for the formation of new hæmoglobin in the blood of moina. Experiments show that a yeast medium needs about 2 to 3 drops of 10 per cent of either ferric chloride or ferrous sulphate to 450 cc to restore the color of moina to that found in nature.

Analysis of ash shows that mungo and soy bean contain more iron than horse manure. The best method so far known for the preparation of a culture medium is to make first a stock solution (experiments 2 and 4), 35 to 40 cc of which are added to 4 liters of water. Stronger concentrations are detrimental to the life of moina, as bacterial masses are formed, interfering with the free movement of the animals and often causing their death. However, in this concentration the quantity of iron is not sufficient to assure the favorable growth of the animal. As in the case of yeast medium, it seems advisable to add iron salt to the culture medium to assure the healthy reddish color in the moina.

The iron content of rice-straw ash is 0.07 per cent (Table 11). Since 8 to 10 grams of straw to 4 liters of water was the optimal concentration for moina culture, the quantity of iron salts in the solution from the straw seemed almost sufficient for the growth of the animals as indicated by the color of the organisms cultured in the infusion.

DISCUSSION

Irregularities in the life span and the number of fecundities are shown in Tables 1 and 2. Some moina mothers died after the fourth brood, while others died shortly after the eleventh (Table 1) and thirteenth (Table 2) broods. There are of course, differences between individuals with regard to the events included in a lifetime. Even under generally uniform conditions, life does not proceed in a uniform, unbroken flow, owing to three factors that break up the uniformity; namely, nutrition, growth, and expulsion of the young. Growth and the expulsion of the

young seem to be exhausting processes. The varying conditions of the intestine alone which is sometimes full and sometimes nearly empty, with large number of young in the egg pouch, or different stages of emptiness of the digestive canal together with the various stages in egg and young development, provide much variety in life processes. In culture there is the interplay between these alternations and the effect of transfer to new fluid. Early or late death depends to a large extent on the degree of exhaustion owing to production of the young and the various nutritive processes, factors which result in high mortality during the later part of the reproductive period or shortly after its close. Some of the animals come through the reproductive process exhausted, and die soon after. Others are hardly damaged at all by this function, and continue to live until they succumb to old age.

Owing to the fixed character of development in moina and the fixed length of the early phases of life, more uniformly defined periods of fecundity and old age might perhaps have been expected. However, both in nature and under laboratory conditions the length of life span and the degree of fecundity vary extremely, and are undoubtedly dependent on environmental conditions.

Throughout the year the sexual or resting egg plays a unique part in maintaining the species in spite of periodically unfavorable weather, as that of the rainy season, which usually begins in June and ends in January. When the rain falls, the water in the pool rises. During hot weather the pool becomes low or dries up completely. The increase of water during rainy season favors the growth of moina, since the population becomes sparse, resting eggs hatch, and new food supplies develop. Due to evaporation of water as well as to the prolificacy of the species, the pools become over-crowded and the food supply exhausted. Males are produced as well as sexual females that drop their ephippial eggs at the bottom of the pool. Some percentage of the eggs hatch out within three or more days and the young die a few days later. The rests of the eggs undergo a resting or latent period in a dry condition. The embryo of the egg remains living in a dry state until more favorable conditions come. It is in this condition that the species survives the dry season.

Crowding is undesirable in mass production, at least when the organisms are artificially cultured. It causes the parthenogenetic females to produce males, and, as a result, the fertiliza-

tion of the majority of the sexual females. A sexual female not fertilized by a male may disintegrate her resting eggs and turn into a parthenogenetic female. Inasmuch as in the majority of cases only one out of two eggs develops in the ephippium, only one offspring may be expected from each brood. Naturally the sexual forms become more preponderant as the culture gets older.

Although the number of generations that may be obtained in one year has not been recorded, since the postembryonic development of the parthenogenetic female of moina lasts 24.29 hours on the average, reproduction may be expected at intervals of from 20.81 to 26.13 hours, and if the animal is maintained in a suitable culture medium throughout the year, the number of generations obtained will undoubtedly be very high. In the case of sexual females the number of generations will be greatly lowered, as some sexual eggs that do not require drying hatch irregularly in the medium and others need to be dried before hatching, depending upon the availability of fresh water, in which case the number of generations will depend upon the environmental factors and the nature of the ephippial eggs.

In culture experiments it was noted that the organisms became whitish not long after they had been cultured on media such as soil-mungo, soil-ricebran, soil-soybean, and yeast, where iron was insufficient for the formation of the new hæmoglobin in the blood of moina. In soil-mungo the average life span is 8.72 days, and in soil-manure it is 12.82 days, while in soil-mungo medium the number of offspring produced by 19 mothers is 2,002, and in soil-manure medium it is 2,486 for 13 mothers. Upon tracing the food nutrients of the two media, it was noted that soil-mungo medium was much inferior to soil-manure medium in iron content in final dilution, which probably accounts for the shorter life span and the smaller number of offspring than those obtained in soil-manure medium.

Yeast is a very nutritious food, as shown in Tables 9, 10, and 11. Analysis (Table 11) shows that it is highly rich in phosphorus, potassium, magnesium, and other constituents, but poor in iron. Owing to its deficiency in iron, which seems to be the immediate cause for the lower vitality and reproductiveness in moina, yeast alone is not good for moina culture, but with the addition of an iron salt in the culture water it may become excellent. This fact may be true not only with regard to yeast medium, but also with regard to soil-mungo medium, soil-ricebran medium, and soil-soybean medium.

Moina thrives well in soil-manure culture medium; the offspring remain reddish, vigorous, and productive even after they have stayed long in the laboratory. Soil-manure medium, when new, contains sufficient nutritious food substances for the host of microörganisms in the medium, which may be used as food by moina.

Undoubtedly food influences the prolific growth of moina. Owing to the abundant fresh food nutrients, the period of greatest reproductiveness occurs when the medium is from 2 to 7 days old. Reproduction reaches its highest level when the food is plentiful, and declines as the food supply becomes exhausted. Exhaustion of food supply and liberation of too much carbon dioxide by moina under crowded conditions may cause the death of practically the entire population of the culture. Since the highest rate of reproduction by parthenogenesis occurs when the culture is sparse and the food supply plentiful, it is necessary in mass culture that the growing population be periodically reduced and rotation methods exercised.

SUMMARY AND CONCLUSIONS

1. *Moina macrocopa* grows abundantly in temporary pools in the vicinity of Manila. It is considered to be the best of all living foods for ornamental fishes, and is reported for the first time in the Philippines.

2. The species under discussion has been variously included in *Daphnia macrocopus* Straus, *Moina rectirostris* Baird, *Moina brachiata* Jurine, *Moina flagellata* Hudendorff, *Moina paradoxa* Weismann, *Moina fisherii* Hellich, *Moina azorica* Moneiz, and *Moina macrocopa* Straus.

3. It is commonly called "daphnia" in Manila. Since the daphnia belongs to a different genus, "moina" should be adopted as the common name of this animal.

4. This waterflea is widely distributed throughout the world, having been reported in Europe, Central Asia, South Africa, United States, South America, Ceylon, and Japan.

5. *Moina macrocopa* lives in fresh water polluted with decomposing material. It may be found throughout the year, as long as water and food are constantly added to the pool.

6. Practically nothing is known concerning the life history of a waterflea in the Philippines. Some phases of the life history of *Moina* have been studied in the United States, Europe, and Japan.

7. There are two types of females, sexual and parthenogenetic, which produce sexual and parthenogenetic eggs, respectively.

8. There are three nymphal instars during the postembryonic development. In individuals cultured in soil-mungo medium the average duration of the first stage is 6.73 hours; that of the second, 8.56; that of the third, 10.50. The total average period from birth to adult brood in soil-mungo medium is 24.89 hours, and in soil-manure 6.53, 8.00, 9.73, and 24.26 hours, respectively.

9. The life span in soil-mungo medium was from 4.23 to 13.30 days, an average of 8.72 days, while in soil-manure the life span was from 7.96 to 14.15, an average of 12.82 days.

10. The number of young produced in soil-mungo medium is 2,091 by 19 parthenogenetic mothers; and in soil-manure medium, 2,486 by 13 mothers. In the total number of offspring produced by 32 mothers the females always outnumbered the males. Difference in the number of offspring is considered to be due to food.

11. The incubation periods of the parthenogenetic eggs were approximated from the last molting of the nymphal instar to the extrusion of the first brood, and from any one brood to the succeeding brood. It is calculated from the laying of the eggs from the germarium to the extrusion of the young from the egg pouch.

12. The male molts twice before it becomes an adult. The first instar takes from 7 to 8 hours and the second from 8 to 9; molting occurs from 18 to 21 hours thereafter.

13. The incubation period of the sexual eggs is variable, inasmuch as some of the eggs may hatch from 2 to 3 days after laying and others require an extended period. All fertilized sexual eggs can be dried and made to hatch within three or more days by thorough soaking in the medium.

14. The molting process was found to be essentially the same in all sexes and stages. The adult females molt shortly after the extrusion of the young or sexual egg, and the males from 18 to 21 hours after the second molt.

15. Copulation occurs after the third molt of the sexual female and the second molting of the male. The process of mating is described.

16. The proportion of sexes in moina is found to be difficult to determine, since it is controlled by environmental factors; namely, food, crowding, temperature, and others.

17. Some enemies of moina are found to be cyclops, *Vortiella*, hydra, Rotifera (*Brachionus rubens*), bacterial masses, molds, and fishes.

18. The method of feeding, which is essentially the same in all stages and sexes, has been described.

19. The organism was able to tolerate a pH range between 5.2 and 7.6 in acid and 7.6 and 9.2 in alkaline. The coloration of the animal was reddish and the rate of reproduction similar, which clearly shows that a favorable hydrogen-ion in a certain medium did not play an important rôle in the coloration and prolific reproduction in moina.

20. Water, the proper amount of oxygen, the range of temperature, the condition of light, the kind of containers used, and the selection of healthy breeders are important factors in laboratory propagation.

21. Food is considered the most important factor in the laboratory breeding of moina.

22. Moina may feed on bacteria in soil-manure and mungo media, and bacteria or yeast plants in yeast, and algæ from fishpond sediment. Thus in nature they may feed on very minute organic matter that may be in suspension in the medium in which they live.

23. In sterilized media with food, principally carbohydrates, moina died in 48 hours without a sign of perceptible growth.

24. The organisms, grew and multiplied in soil-soybean, soil-ricebran, soil-mungo, and yeast media, but lost their vigor by gradually turning from reddish to whitish and declined in reproductiveness. In soil-manure medium moina grew normally, retaining their vigor even after they had been cultured for a long time in the laboratory. In rice-straw infusion moina seems to grow normally but becomes entangled by molds which grow out of the straw.

25. With the use of horse-manure medium and by following the culture method herein described, mass production is possible. For culturing moina as food for large number of fishes, the tank in which the animals are cultured should be large enough to accommodate the quantity of moina needed for the number of fishes to be fed. Inasmuch as the exhaustion of the food supply occurs at about the fifth day after introduction of the organisms into the culture medium, the entire change of the medium and rotation become necessary to maintain a continuous supply of the organisms.

26. Since in soil-soybean, soil-ricebran, soil-mungo, and yeast media, the animals gradually turn from reddish to whitish, analyses of food values of the different materials were made. The loss of color was found to be not due to deficiency in fats, proteins, and carbohydrates, but to the lack of iron salts in various concentrations of the media used.

27. Soil-manure is considered to be the best of all the media tested, because, in it, the animals remained in normal condition; the medium may be sufficiently highly concentrated to provide iron in solution for the assimilation of the unicellular organisms serving as food for moina.

ACKNOWLEDGMENT

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ILLUSTRATION

[Drawings by V. V. Marasigan and H. T. Castelo.]

PLATE 1

FIG. 1. Ephippial case in lateral position, showing reticulation and two eggs; about × 12.

2. First nymphal stage; about × 12.

3. Second nymphal stage; about × 12.

4. Third nymphal stage; about × 12.

5. Adult sexual mother moina, showing an ephippial case and eggs in the egg pouch; about × 12.

6. Parthenogenetic mother moina bearing parthenogenetic eggs in the egg pouch; about × 12.

7. Parthenogenetic mother moina with young about to be extruded and showing *se*, setæ; *cl*, claw; *ft*, feet; *ca*, carapace; *œ*, œsophagus; *ant*, antennule; *b*, brain; *e*, eyes; *ce*, cæcum; *an*, antenna; and *r*, ramus.

8. Postabdomen showing ten ciliated spines besides bident and a pair of claws with longitudinal laterally arranged rows of hairs; about × 47.

9. Male moina; about × 12.

10. Antennule showing the number of hooks, and two sensory setæ in the middle; about × 47.

11. First foot of a male; about × 23.

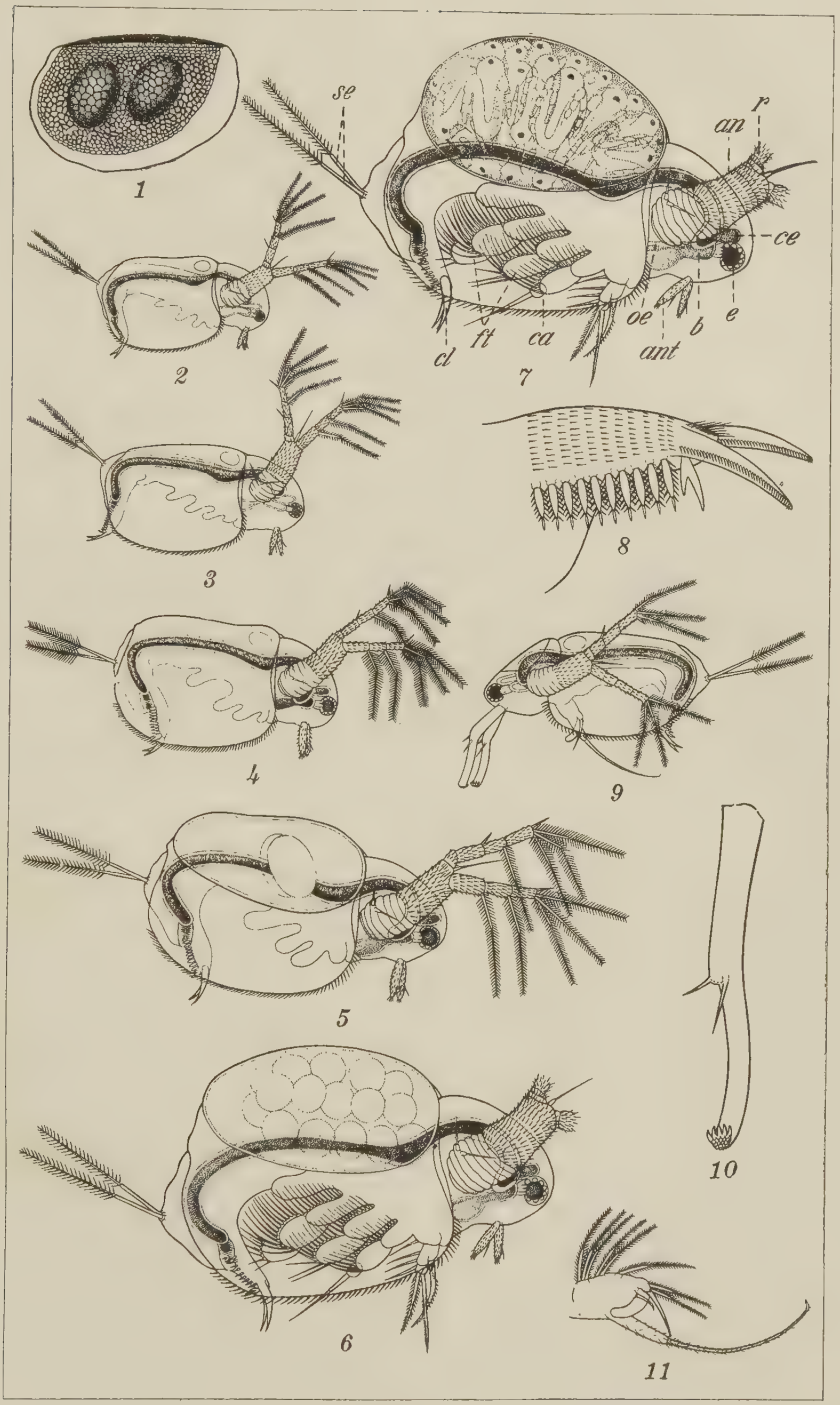

PLATE 1.

BOOKS

Acknowledgment of all books received by the Philippine Journal of Science will be made in this column, from which a selection will be made for review.

RECEIVED

AVERILL, L. A. Adolescence; a study in the teen years. Boston, Houghton Mifflin co., 1936. 496 pp. Price, $2.25.

BELSHAW, H. Recovery measures in New Zealand. Wellington, N. Z. Inst. of Pacific relations, 1936. 61 pp. Price, paper, $0.25.

The British plastic year book, 1937; the handbook and guide to the plastics industry. London, The Proprietors: Plastics press ltd., 1937. 602 pp., illus. Price, 15s.

CLARK, A. H. Crinoidea. [British museum (Natural history) John Murray expedition, 1933–34, Scientific reports, v. 4, no. 4.] London, Printed by order of the Trustees of the British museum, 1936. 22 pp., plate. Price, 2s.

COCKERELL, T. D. A. African bees of the genera Ceratina, Halictus, and Megachile. London, Printed by order of the Trustees of the British museum, 1937. 254 pp., illus. Price, 15s.

FUNK, CASIMIR, and H. E. DUBIN. Vitamin and mineral therapy; practical manual. New York, U. S. Vitamin corporation, 1936. 94 pp.

MARTINDALE, WILLIAM, and W. W. WESTCOTT. The Extra pharmacopœia. Vol. 1. 21st ed. rev. London, The Pharmaceutical press, 1936. 1182 pp. Price, 27s. 6d.

ROBERTS, HARRY. Euthanasia and other aspects of life and death. London, Constable & co. ltd., 1936. 278 pp. Price, 7s. 6d.

TALBOT, G. A monograph of the Pierine Genus Delias. Pt. VI. London, Printed by order of the Trustees of the British museum, 1937. 656 pp., plates. Price, £1.5s.

VATSYAYANA, MALLINAGA. Kama Sutra: the Hindu science of love. Tr. from the Sanskrit by Sir Richard Burton. The Doctor as marriage adviser, by Max Hodan. Introduction by H. H. Ewers. Illustrated by Mahlon Blaine. New York, The Medical press, 1936. 127 pp., illus. Price, $2.

WATERSTON, JAMES. Fleas as a menace to man and domestic animals. [British museum (Natural history) Economic ser. no. 3. 3d rev. ed.] London, Printed by order of the Trustees of the British museum, 1936. 20 pp., illus. Price, 4p.

WHEELER, J. F. G. Nemertea. [British museum (Natural history) John Murray expedition, 1933–34. Scientific reports, v. 4, no. 3.] London, Printed by order of the Trustees of the British museum, 1936. 8 pp., text figs. Price, 1s.

The Wine book of South Africa. Stellenbosch, South Africa, "Wine and Spirit" Publishers, 1936. 224 pp., tables, map. Price, 5s. 6d.

REVIEWS

Useful Drugs; a Selected List of Essential Drugs with Brief Discussions of Action, Uses, and Dosage. By Robert A. Hatcher. 10th ed. American medical association, Chicago, 1936. 240 pp.

The existence of a great number of drugs and their preparations prompted the American Medical Association to publish this volume which is now in its tenth edition since 1913. The list consists of a number of important drugs taken from the United States Pharmacopœia and from the New and Nonofficial Remedies which are known to posses established therapeutic value. The present edition, a thorough revision of the 1934 edition, is based on the United States Pharmacopœia XI and the New and Nonofficial Remedies, 1936.

The definitions, synonyms, properties, incompatibilities, action and uses, and dosage of drugs are treated in convenient form for ready reference.

The student as well as the practitioner will find this book very useful—P. V.

The Extra Pharmacopœia. By William Martindale. Vol. 1. 21st ed. rev. The Pharmaceutical press, London, 1936. 1182 pp. Price, 27s. 6d.

For the second time this valuable reference book is being published in two volumes by the Council of the Pharmaceutical Society of Great Britain. The present volume corresponds to the first part of this "comprehensive summary of the composition and applications of the multitude of old and new, official and proprietary substances about which the doctor or the pharmacist may require information." The physician can obtain from it the necessary information on compounds and preparations of wide use in therapeutics, whereas a general and practical knowledge of the constitution and properties of the chemical, animal and vegetable drugs generally encountered in the compounding and dispensing of medicines is made available to the pharmacist. The valuable notes on the composition of medicinal agents and the practical observations on the results of their application included in this reference are of particular interest to the clinicians.

The revision of the volume under review was carried out under the direction of C. E. Corfield, editor of the British Pharmaceutical Codex, H. Treves Brown, his research assistant, and S. L. Ward, the abstractor. This circumstance explains why the present "Extra Pharmacopœia" is considered a complementary volume to the British Pharmaceutical Codex.

The compilers of the present edition have not failed to examine the new pharmacopœias and formularies that were issued since the publication of the twentieth edition. Consequently they were enabled to bring the materials in line with the requirements of the United States Pharmacopœia XI, the Swiss Pharmacopœia V, the Danish Pharmacopœia, 1933, the addendum to the British Pharmacopœia, 1932, and the National Formulary VI, 1936, besides the British Pharmaceutical Codex, 1934.

One of the important features of the book is the inclusion of information relative to proprietary medicine as regards the name, the source of the article, and other data regarding the preparation. As stated in the preface "the inclusion of names of these proprietary forms of a drug is an important feature of the Extra Pharmacopœia, and provides practically the only concise information available to the doctor by which he can discover whether many of the so-called ethical proprietaries are new products of the research laboratory, or simply older chemicals or preparations making a new appearance in a modified form."

The obtention of the volume under review will certainly be an important addition to a medical and pharmaceutical library.—P. V.

Sex Education. By Maurice A. Bigelow. The American social hygiene association, New York, 1936. Rev. ed. 307 pp. Price, $1.

This book is well written and instructive for the general reader. It presents in simple language the problems of sex education, and the need for their clear understanding among teachers and parents especially. The problems of sex education, including that of who are desirable teachers and the dangers to be avoided, are clearly outlined. Besides sex instruction, it deals with special social problems which should be adequately and sanely met by young people. It is a worth while book for those interested in social conditions and for young people desiring guidance in their social life.—U. D. M.

Cancer Commission Committee Studies. By the California Medical Association. J. W. Stacey, Inc., San Francisco, California, 1936. 123 pp. Paper.

This is only a reprint of the series of studies, reports, conclusions, and recommendations which the above Commission has been publishing in California and Western Medicine during the past five years.

It deals with radiology, gynæcology, tumors, breast tumors, skin and mouth tumors, eye, ear, nose, and throat tumors, genito-urinary tumors, chest tumors, bone tumors, connective-tissue tumors and leukæmias, tumors of the central nervous system, gastro-intestinal tumors, cancer of the rectum, anus, or rectosigmoid, and thyroid tumors. There is an appendix on lymph glands in cancer of the lip, tongue, and mouth, and their treatment. Each group of tumors is the subject of a report of a separate committee. The conclusions and recommendations of the commission are fully orthodox and in line with the accepted views on the prophylaxis and treatment of cancer.—C. R.

Tumors of Bone (Including the Jaws and Joints). By C. F. Geschickter and M. M. Copeland. The American Journal of Cancer, New York City, 1936. Rev. ed. 817 pp., illus. Price, $6.

This is a monumental monograph on bone tumors, including the jaws and joints, written on material from the Department of Surgery of the Johns Hopkins Medical School. The text proper is presented in 25 chapters, the subject of each chapter being developed from the standpoint of clinical features, gross pathology, microscopic features, observations or variants, histogenesis, roentgenographic features, etiology, and prognosis, all of which are briefly summarized at the end and furnished with a bibliography. The various features are also presented in a condensed tabulated form.

The originality of the book makes a critical review somewhat difficult, but some of the authors' most important findings are:

Permanent cures in primary chondromyxosarcomas average about 12 per cent. The usual clinical course of the primary form is a fatal termination in the space of 20 months.

Permanent cures in the secondary chondromyxosarcomas average about 27 per cent. The disease runs from 6 to 8 years.

Permanent cures in sclerosing osteogenic sarcoma are obtained in approximately 25 per cent of patients. Prompt resection or amputation but not deep X-ray or radium therapy is advised. The diagnosis of all three of these conditions depends primarily upon the clinical picture rather than upon the X-ray.

An enlargement of the regional lymph nodes may occur with malign bone tumor but does not indicate metastases to these nodes. It is always a mistake to make a biopsy on the regional nodes for any type of bone lesion, unless metastatic involvement of the bone itself by cancer or by a malign node is suspected.

The great majority of permanent cures have resulted from resection or amputation, with or without pre- or post-operative irradiation. Biopsy, preceding resection or amputation by an interval of days, is not detrimental to cure. The element of risk involved in a properly performed biopsy of the bone which is followed in an interval of fourteen days or less by amputation is so small that one is justified in taking this risk and in sending the X-ray films and sections for consultation. Biopsy may unfavorably influence cure by the escape of fluid into the wound.

The most highly differentiated and the most curable form of bone sarcoma is the periosteal fibrosarcoma.

The foreword dealing with interpretation of clinical findings by Dr. Dean Lewis is very illuminating, as are the 22 rules on diagnostic and therapeutic procedure for bone lesions by Dr. Joseph Colt Bloodgood, which must be read in order to be appreciated; they deal with biopsy—when to and when not to perform it, irradiation, palpation, X-rays, search for infection everywhere, blood Wassermann, blood count, urine or Bence-Jones bodies, blood sugar, fever, blood chemistry, and metabolism test.—C. R.

Leçons de Physiologie Medico-Chirurgicale. By Leon Binet. Masson & Cie., Paris, 1935. 244 pp., plates, figs. Price, 40 fr.

This book contains the first series of weekly conferences given by the author since he assumed the chair of Physiology at the Faculty of Medicine at Paris, with the collaboration of other French clinicians, notably Jean Gosset and M. Kaplan. It includes a wide range of subjects, such as resuscitation, the physiopathology of arterial emboli, arterial atheroma, pneumothorax among the tuberculous, pathogeny and treatment of gastro duodenal ulcers, colibacillosis, syndrome and medical treatment of intestinal obstruction, acute hæmorrhagic pancreatitis, functional testing of the spleen, experimental therapy of acute nephritis, diseases of the spinal cord, the great painful syndromes about the face, spontaneous and artificial rickets, and the antirachitic substances, the thymus and sexual glands and the results of thymopotherapy, the biologic diagnosis of pregnancy, snake venoms, and oil injections. As is to be expected, the subjects are dealt with chiefly from the standpoint of applied physiology, and done in the lucid manner common to many French masters, they cannot fail to interest the physician who must ever keep himself close to the sources, among others, the physiologic background.—C. R.

The Surgical Technic of Abdominal Operations. By Julius L. Spivack. S. B. Debour, Chicago, 1936. 718 pp., illus., figs. Price, $10.

The surgical technic of abdominal surgery contained in one single volume affords medical students an easy and an ever-ready guide, doing away with the necessity of having to consult the usual set of several volumes in which abdominal surgery is only a part.

The chapter dealing with general surgical technic is very illuminating. The important anatomical points in the surgery of the different abdominal organs, with the additional historical background in the evolution and development of technic especially with reference to eminent researchers and surgeons, not only in America but also in continental Europe, is very scholarly, giving as it does to the reader a comprehensive grasp and understanding of the difficulties and shortcomings heretofore met with and the corresponding mistakes overcome and corrected in the introduction and presentation of better technic, together with the still considerable improvement remaining to be accomplished.

The different technics in abdominal surgery are of course found in standard sets, like Bickham; but in this volume they are more concise, with their presentation aided by classical pictures and sketches that drive home the points of simplicity, the minimum of effort on the part of the operator and his assistants, and the optimum of results.

Special mention should be made of the part on the surgery of the stomach, intestines, herniæ, and appendix, in which similar operations and technics are actually being carried out in the Department of Surgery of this Hospital.

The bibliography affords the reader a ready reference to old and new literature dealing with kindred topics.

In recapitulation, the surgical technic of abdominal operations contained in this book by Professor Spivack, a new man in the surgical world, is very useful to students and practitioners alike; to the student because of the accessibility in one copy of his limited library, and its concise and brief form without the danger of his being lost in a haze of intricate surgical elaborations; and to the practitioner for the review of the important steps in the operation he is about to perform. Instructors and professors of surgery, who always prefer to give the widest scope of lectures and demonstrations in teaching the regional surgery of the ab-

domen within the limited instructional time alloted them in the curricular course, should welcome this volume especially.

—B. R. D.

Spider Wonders of Australia. By Keith C. McKeown. Angus & Robertson Ltd., Sydney, 1936. 270 pp., illus., plates. Price, 6s.

This book is divided into 18 chapters. According to the preface, the author was induced to write the book in view of the many inquiries that have been received by the Australian Museum of Sydney about spiders. The epilogue deals with an ancient Greek maiden Arachne who because of the wrath of one of the gods was changed into a spider. From this Greek legendary character the name of the class Arachnida was derived.

In the introductory chapter the author has cleverly chosen examples of spiders the habits of which vividly portray to the reader "the strangeness of the spider world." He states that in Australia there are some 1,200 species known and that possibly as many remain to be described and classified. Due to popular ignorance and prejudice, spiders are persecuted and are killed unnecessarily. The author enters a plea of a kinder treatment of the majority of these curious, and, in the main, useful and interesting animals. Of the large number of species of spiders in Australia only three are definitely known to be poisonous to man, and this black list includes the so-called Redback or Jockey Spider, *Latrodectes hasselti,* which is also present in the Philippines.

In Chapters 2 to 15, inclusive, the author writes clearly and interestingly of the habits and ways of life of some of the curious spiders of Australia. In this work the author has largely drawn upon his notes and observations, extending over a number of years, for, as he points out in the preface, very little has been written about the habits of Australian spiders. Pertinent observations from the few scattered writings of previous authors are quoted.

The book is interesting reading from cover to cover. Although it is all about Australian spiders, yet the knowledge furnished therein should be of value to persons in other lands undertaking studies on spiders and their habits. The fact that the writer is a scientist, an entomologist who has previously published a similar book entitled, "Insect Wonders of Australia," increases the reader's faith in the accuracy of the observations recorded in this new book—F. Q. O.